

MICHIGAN
STATE AGRICULTURAL COLLEGE
EXPERIMENT STATION

DEPARTMENT OF BACTERIOLOGY AND HYGIENE

AÆRATION OF MILK

(Thesis presented for the degree of Doctor of Philosophy, University of Michigan.)

BY

CHARLES E. MARSHALL.

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THE AËRATION OF MILK*.

CHARLES E. MARSHALL.

Although practical aëration of milk may be traced back to an indefinite past, where it is simply mentioned and recognized, there has never been any plausible explanation or demonstration of the results claimed for it. Moreover, its application has always been conspicuously limited until within very recent years, and so far as its value is concerned at the present time, nothing has been satisfactorily established regarding it, other than the belief that it eliminates animal odors and removes, to a certain extent, tainted conditions of milk resulting either from physiological metabolism due to ingestion of aromatic food substances or produced by the growth of saprophytic bacteria in milk after the milk has left the udder of the cow. Even these animal odors and taints have been more or less undefinable and immeasurable; only daily practices in dairy operations have indicated and established beyond a doubt that both of these are greatly lessened by proper aëration.

Many other beliefs have been associated with or attributed to the process of aëration, such as, the production of more cheese and better cheese, the production of more butter and better butter, the more rapid rising of the cream, the more rapid churning of the cream, the better keeping qualities of the milk and butter—all of these advantages have been assigned to aëration over the non-aëration of milk.

I. REVIEW OF AËRATION OF MILK.

Studying from the standpoint of the dairyman, Willard (1), Arnold (2), Lewis (3), Cooke (4), Wing (5), Plumb (6), Dean (7), and the Danes (8) under Storch have contributed to our knowledge of its application as it is at present practiced, but all of their work fails to explain the process itself or to state what aëration is. They do not throw light on why

*Thesis presented for the degree of Doctor of Philosophy, University of Michigan.

(1.) Willard's Practical Dairy Husbandry, 1871, p. 183.

(2.) Loc. cit.

(3.) U. S. Agric. Report, 1874, p. 397.

(4.) Ver. Exp. Stat. Report, 1892, p. 123.

(5.) Cornell Exp. Stat., Bul. 39.

(6.) Purdue Exp. Stat., Bul. 44.

(7.) College Reports (Guelph, Ont.), 1898, 1899, 1900, 1901.

(8.) 48 de Beretning fra den Kgl. Veterinaer-og Landbokijskoles Laboratorium for Landkonomiske Farsog.

aëration may answer any of the above questions named. An attempt is made to demonstrate whether butter made from aërated milk is better than butter made from non-aërated milk, whether more cheese has been made from milk that has been aërated than from milk that has not been aërated. By these methods, perhaps, some valuable points have been settled, and still the future may change the character of this work wholly, since it is true they have not answered the question, "What is aëration?" or more explicitly, "were they really working with aërated milk or non-aërated milk?"

It seems pertinent at the start, that before we can understand or explain aëration of milk it is necessary we understand what aëration is, and we know whether we are producing aërated milk or not. All the practical experiments executed have been with aëration and non-aëration as they are known to us from present methods alone. The difference between the two may not be so marked as suspected and for this reason fail in producing the most noticeable effects. The starting point, non-aëration, must be first established and from that our conclusions should, in a way, be drawn; that is, we must determine any change in the carbon dioxide and oxygen content, with a decrease in the former and an increase in the latter as a process whose final limitations are not known or influences realized.

It is regretted that the value of our present aërating methods cannot be stated more exactly at this time, rather than depend upon the virtually negative results obtained by the individuals named in their endeavor to secure some practical explanation through their practical experiments. We hope to make this our next task; for the present we must content ourselves with the references to be gained from the evidence at hand. The aërating methods now in vogue in dairy operations may or may not yield results. It is not necessary to say whether these methods are effectual or not; for our purpose their value cannot be determined until each method has been worked out with pains-taking care. As soon as there has been established a non-aërated milk and a satisfactory aërated milk, then we may hope to reach some definite conclusion in its application to the dairy. Before this is done, the one hope that can be held out is that some of the aërating methods now employed may be giving some advantages over the non-aërating methods besides those which are recognized in the removal of odors and taints in a slight degree.

The work which follows has for its immediate aim the demonstration of what constitutes non-aëration and aëration and how it may influence the condition of the milk itself and the germ content of the milk. After getting into the work it has been found necessary to eliminate for the present several side-problems which bear directly upon aëration and confine ourselves to the main line planned at the start. It should be noted, therefore, that all the aspects are not to be satisfactorily considered in this article, but rather, that a path has been hewn out of an unknown forest of doubt and ignorance which we may follow with profit in the future and may eventually be led to the solution of the whole question.

II. REVIEW OF THE GAS-CONTENT OF MILK.

The first problem which confronts the experimenter is to obtain a knowledge of the gas-content of the milk before exposure to the air. Dr. Felix Hoppe (9), in 1859, made a study of the gases of milk from a goat.

His analyses are as follows:—

First Analysis.

Carbon dioxide	9	parts.
Oxygen	9.57	"
Nitrogen	8.43	"

This trial should be regarded in the nature of a preliminary test.

In securing the milk he drew it into a funnel contiguous to the end of the teat of the goat, and conducted the milk into a flask from which the air was expelled as the milk filled the flask. To this method of securing the milk, there is an objection because the milk had been exposed to the oxygen of the air, not only in its agitation while passing along the tube in contact with the air, but also in the surface contact with the oxygen in the air in the flask itself.

Second Analysis.

Carbon dioxide	55.15	per cent.
Oxygen	4.29	" "
Nitrogen	40.56	" "

He secured 12.08 volumes of gas from 402 volumes of milk measured at 0° and 1 M pressure.

With this analysis the same criticism can be made as with the first analysis. He used the funnel but improved the design of the flask. When these analyses are compared, therefore, with the analyses of the gases from the milk immediately after milking, it will be seen at once that he has reached practically the same results; consequently his analyses are no more than the analyses of gases from milk immediately after milking. Without reference to his method of securing the gas, which is also open to condemnation, it follows that this work may be disregarded and is no criterion to follow when considering the gas-content of milk as it comes from the udder of the cow.

Stechnow (10) adopted another method of securing the milk. Olive oil, the best obtainable, was used to cover the milk. He inserted the end of the teat into the oil, then milked, thus maintaining a layer of oil over the surface of the milk. This, doubtless, had an influence in restraining the effect of the oxygen in the air by keeping the air away from the surface of the milk and also reducing the oxygen content which the milk as it passed in a stream from the teat of the cow to the receptacle

(9.) Arch. f. path. Anat. and Physiol. und f. klinische Medicin, 1859, Bd. 17, 5. 417.

(10.) Zeit. f. rat. Medicin von Henle u. Pfeuffer, Bd. 10, S. 285, 1861.

necessarily acquired. An objection, however, should be offered to the use of oil for the exclusion of oxygen, because it is a well known fact that oil contains oxygen in the form of super-oxygenated compounds, and also probably as free oxygen. In securing the gases from the milk a mercury pump was used as seems to have been the general practice with Stechnow and those who followed him, to which little criticism can be offered as viewed from the results obtained.

His analyses are as follows:—

First Analysis.

Carbon dioxide	77.50 per cent.
Oxygen and nitrogen.....	22.50 “ “

He obtained 7.29 c. cm. of gases from 100 c. cm. of milk and employed for his analysis 67.5 c. cm. of milk.

Second Analysis.

Carbon dioxide	81.06 per cent.
Oxygen	1.93 “ “
Nitrogen	17.01 “ “

He obtained 8.29 c. cm. of gases per 100 c. cm. of milk. He used 297.5 c. cm. of milk.

Third Analysis.

Carbon dioxide	75.11 per cent.
Oxygen	4.79 “ “
Nitrogen	20.10 “ “

Total gas is equal to 6.67 c. cm. per 100 c. cm. of milk. Amount of milk used 256.3 c. cm.

Pfütter (11), believing that Stechnow was open to error in his method of securing the milk from the udder by shielding it as he did from the air by means of oil, attempted to overcome it by another device for conducting the milk from the udder to the receptacle without exposure to the air at all. To do this he employed a piece of rubber, fitting over the teat and udder of the cow as a glove would fit upon the finger; and, by means of a narrow rubber tube and glass tube was able to lead the milk into a vessel of mercury and collect it over mercury. He first expelled the air from the tube by milking for a time, filling the tube with milk, and then placing the free end of the tube under the mercury in the container. This course may be open to criticism in that there would be some leakage about the surface of the teat over which the rubber was fitted, but the analyses of Pfütter indicate that he was not troubled very much by the possible exposure to the air from this source and that the milk secured by this method was practically free, if not wholly free, from air.

(11.) Pfütter's Arch. f. Physiol., Bd. II, S. 166-173, 1869.

Further than this, his results are as satisfactory as any of the results obtained when other methods are employed for securing the milk, and which, in our case, we believe, did not allow of any exposure to the air at all. This will be shown later.

Pflüger's analyses are as follows:—

First Analysis.

Carbon dioxide	90.45	per cent.
Oxygen	1.19	“ “
Nitrogen	8.36	“ “

Amount of milk used was 60.77 c. cm. Amount of gas obtained per 100 c. cm. was 8.47 c. cm. No trace of gas was obtained after one hour.

Second Analysis.

Carbon dioxide	87.16	per cent.
Oxygen	1.06	“ “
Nitrogen	11.78	“ “

Amount of gas per 100 c. cm. of milk was 8.49 c. cm. Amount of milk used 77.319 c. cm.

The analyses of all these men were made directly after milking, and did not extend over a time sufficient to warrant fermentation.

Pflüger was of the opinion that he had secured all the gas possible within one hour's time and made no allowance for a slow generation of gas after the hour when subjected to high pressure. This is not in accord with our experience, for after the first ebullition of gas which takes place early in the pumping process there follows a slight development of gas until fermentation sets in, when there is a marked increase.

III. DESCRIPTION OF APPARATUS.

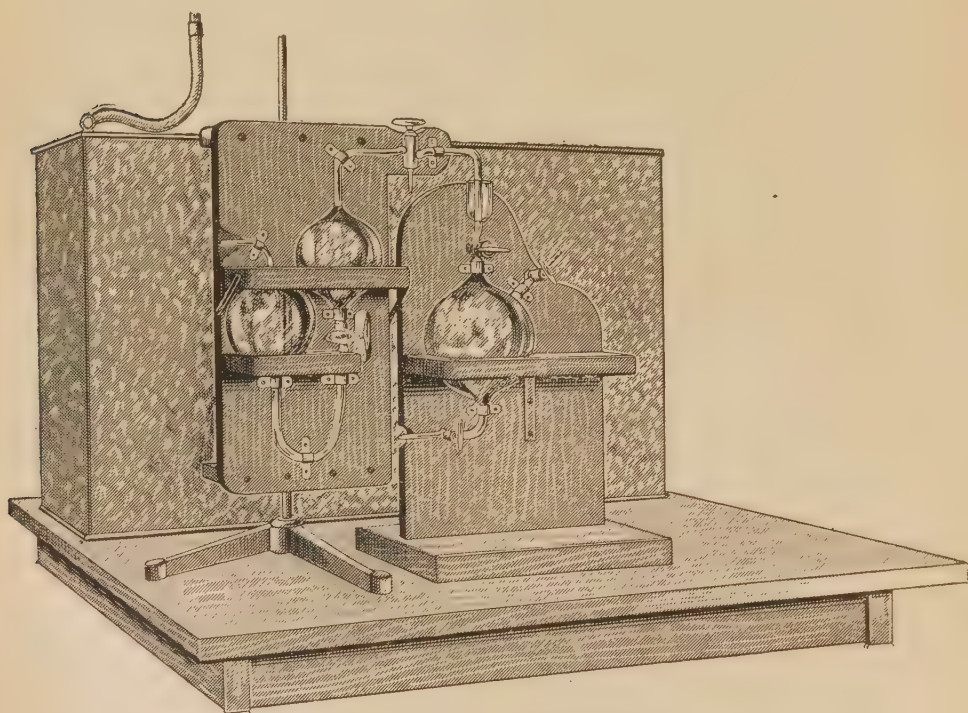
For our experiments we departed from the methods used by Hoppe, Stechnow, and Pflüger, in that we endeavored to eliminate any possible chance of exposure of the milk to the air. Pflüger's method, the best previously adopted, is open somewhat to criticism in that there is a possible chance of the milk coming in contact with the air or being more or less churned with traces of air. His analyses, however, indicate that errors of this kind scarcely made any difference, in fact, he seems to have obtained about the same amount of oxygen in his experiments as were obtained in ours. In order to secure the milk from the udder of the cow without any exposure to the air we employed a milking tube 6.6 cm. long attached and fitted to a piece of pressure tubing 80 cm. long and 5 mm. in diameter. The walls of this tube were 4 mm. thick. Over the aperture of the milking-tube was placed a piece of thick rubber tubing of small caliber so that it just fitted the tube. This served as a slide valve in closing and opening the apertures. The other end of the

rubber tube was attached to a glass tube, 5 mm. bore, containing a three way stop-cock and this in turn was attached to the receptacle used as a container for the milk by means of a short piece of pressure tubing. This will now be described.

The container for the milk is of the nature of a large bulb of heavy glass fixed securely to a standard of wood. It has three openings all of which are closed by means of a glass stop-cock at will. One of these openings is at the bottom of the bulb, another directly above at the top and the other a side arm on the right shoulder. The top arm consisted of a capillary tube containing a three way stop-cock an inch above the bulb, and above this a cup and the male portion of a mercury overlapping glass connection. The bottom opening and right shoulder opening were tubes 5 mm. in diameter, fitted with two way stop-cocks. When ready for use, this bulb, with a capacity of 562 c. cm., was filled with dry mercury. The side arm connected with the tube extending to the milk-duct of the udder was filled with mercury as far as the three way stop-cock; the remaining distance was filled completely with boiling water, driving out all the air, and when driven out, all of the apertures of the milking-tube were closed by means of a sliding rubber valve. When ready for drawing the milk, the tip of the milking-tube was carefully inserted into the milk-duct of the udder, and as the teat was pushed on the rubber valve over the apertures in the tube was pushed down, thus allowing the milking-tube to be inserted without the escape or the entrance of any air. When all was in readiness the three way stop-cock was so turned as to allow the water to run out and the milk take its place; when the three way stop-cock was again so turned that the milk would run in the direction of the container. The stop-cock of the side arm of the container was also turned on. This made, therefore, a direct connection from the udder to the container of the milk without the presence of any air whatever. To draw the milk the stop-cock of the lower-arm of the container was turned so as to allow the mercury to escape, drawing the milk from the udder as normally as it was possible. With the running out of the mercury the milk entered and filled all parts of the container, so that when the mercury had all escaped and the lower stop-cock turned off, the receptacle was completely filled without even traces of air.

As soon as the container was filled with milk direct from the udder it was taken to the laboratory and immediately connected, by means of the mercury valve of the top arm, to a modified Hempel pipette specially made for the purpose in which all rubber connections were excluded. The bulb of the pipette, the nearest the milk container when connected, was so made as to be above the second bulb, next to the Geissler pump. From the very top part of the bulb next to the container extended upright 5 cm. in length, a capillary tube; this then turned to a horizontal position for 10 cm.; finally turned downward in a vertical direction and connected with the mercury valve of the container, previously described, by means of its female portion. In the middle of the horizontal portion of this capillary connecting tube was another three way stop-cock which facilitated the filling of the tube with mercury. Therefore the container could be directly connected with the pipette through the capillary tube by means of the mercury connection. Before beginning to pump the gas off, the upper bulb of the pipette and the capillary arm

were filled completely with dry mercury by means of the three way stop-cocks. This was done so effectually that no traces of air were present. A Geissler's pump was then attached to the upper stem of the lower bulb of the pipette, the air was removed, thus creating considerable pressure upon the mercury in the pipette. The stop-cock in the upper arm of the container was then turned so that the gas from the milk could escape and be drawn over into the upper bulb of the pipette and thus collected there. When the operation of pumping was completed the stop-cock in the upper arm of the container was again turned off. To this three way stop-cock was attached a Hempel burette, filled with mercury, by means of pressure tubing also filled with mercury. (It is understood that all rubber tubing connections throughout the entire experiment were further safeguarded by wiring.)



As soon as the pressure of the pump was released and reduced to normal conditions all of the gas was drawn over through the capillary tube and three way stop-cock into the burette where it was held for analysis. In carrying out the analyses of the gases we used Hempel's methods entirely, employing his pipette and White's (12) burette corrected for temperature and barometric pressure. The carbonic acid gas was absorbed by means of potassium hydroxide, the oxygen was absorbed by means of alkaline pyrogallate and what remained was considered as

(12.) Jour. of the American Chemical Society, Vol. XXII, No. 6, 1900.

residual gas. In the case of previous experimenters, the residual gas has been called nitrogen, to me a very presumptuous proceeding, inasmuch as there is apparently much concealed in the residual gas concerning which we know little. In the lower curves of the pipette a little mercury was placed to act as valves to prevent diffusion. The three way stop-cock of White's burette enabled us to fill the connecting tubes between the pipette and burette completely. By this means all errors were reduced to a minimum.

The milk used in these experiments came from one cow, and usually from a single quarter. In none of these analyses did the milk come from more than a single quarter, since a quarter contained sufficient milk to fill the container and the possibility of air entering in changing from one quarter to another was thus avoided. It was found in several cases, too, in our work that should the milk become exhausted in a single quarter the tissues closed over the apertures of the milking-tube and prevented the further flow of the mercury; this further demonstrates that no air could possibly enter by means of the apertures in the milking-tube. The whole apparatus including tubes, container, connections, and pipette was severely tested by several preliminary trials from the starting of the milk from the udder through to the securing of the gas from the milk by pumping. Everything had been verified by these preliminary trials. (In all of our gas analyses the barometric pressure has been reduced to 760 mm. at 0° C, unless otherwise stated.) (A uniform pressure during pumping was maintained by a large galvanized iron tank filled with water in which the entire apparatus was placed.)

IV. ANALYSES OF THE GAS-CONTENT OF MILK BEFORE EXPOSURE TO AIR.

The following six samples were run through and the gases analyzed with these results:—

TABLE I.

Sample.	I.	II.	III.	IV.	V.	VI.
Date.....	Dec. 9th.	Dec. 11th.	Dec. 13th.	Dec. 14th.	Dec. 16th.	Dec. 17th.
Began milking at.....	8:20 a. m.	8:05 a. m.	8:20 a. m.	8:20 a. m.	8:15 a. m.	9:00 a. m.
Finished milking at.....	8:35 a. m.	8:20 a. m.	8:35 a. m.	8:35 a. m.	8:30 a. m.	9:15 a. m.
Began pumping at.....	8:55 a. m.	8:40 a. m.	8:55 a. m.	9:00 a. m.	8:45 a. m.	9:40 a. m.
Completed pumping at.....	9:55 a. m.	9:40 a. m.	9:55 a. m.	10:00 a. m.	9:45 a. m.	10:40 a. m.
Temperature of water in tank....	38°	38.5°	37.5°	37.5°	37.5°	37.5°
Barometric pressure in mm.....	688.63	695.265	682.937	686.93	694.937	690.937
Total gas obtained in c. cm.....	6.16	4.4	4.218	5.342	5.592	4.256
Free carbon dioxide in c. cm.....	4.96	3.44	3.447	4.3788	4.4825	3.667
Per cent. of carbon dioxide.....	80.58	78.35	81.73	81.97	80.16	86.19
Oxygen in c. cm.....	.117	.135	.1134	.1143	.0894	.09
Per cent. of oxygen.....	2.88	3.09	2.69	2.14	1.6	2.12
Residual gas in c. cm.....	1.02	.816	.658	.8489	1.02	.499
Per cent. of residual gas.....	16.54	18.56	18.27	15.89	18.24	11.71

The above six analyses furnish an average which is lower than that of Pflüger, but this does not appear to detract from the value of the work of either after considering the possible variation occurring in the gas-content of milk from the same quarter of the cow. The amount of carbon dioxide obtained in the above analyses is in percentages,

80.58, 78.35, 81.73, 81.97, 80.16 and 86.19.

The averages of these percentages is $81.49\frac{2}{3}$ per cent.

Pflüger obtained in his analyses 90.45 per cent and 87.16 per cent.

The average of Pflüger's analyses therefore would be 88.80 per cent.

Considering the extremes of the six analyses there exists a difference of 7.84 per cent. In Pflüger's the difference is 3.28 per cent. Between the two sets of averages, Pflüger's and ours, there is 7.30 per cent difference. Thus the possible variation in six analyses is greater than the difference between the averages of the two sets of analyses. The uniformity obtained in the six analyses also indicates that there can be no serious discrepancies in the analytical work, and it is difficult to say how much variation Pflüger might have had, had he made six analyses instead of two; furthermore, there is probably as much variation in the gas-content of milk as there is in other constituents. This fact was brought out plainly in some of the preliminary tests which are not cited here because those tests served only to pave the way for these six analyses and to develop those points necessary for recording the systematic work. Pflüger further does not state the temperature at which the gas was pumped from the milk nor does he enter specifically into a discussion of the influences which might alter further the gas-content of milk.

He obtained a larger amount of gas from milk than we obtained. This alone may answer why more carbon-dioxide was secured by him. In two of our preliminary tests we first pumped off the gas, which came with ease, collected it in a burette, and then began pumping again, when we obtained a larger percentage of carbon dioxide than we did in the first pumping; however, we did not carry out this work repeatedly, therefore cannot state with any degree of assurance that this will always follow. It was not our purpose to ascertain the amount of gas that may be obtained from milk, but rather to secure a qualitative estimation of any changes that may occur in milk under aëration.

To do this it became necessary to hold to the same time and temperature and pressure, as far as possible, while pumping the gas from the milk. After trying the temperature of the room in several tests, about 22° centigrade, the conclusion was reached that the yield in amount of gas in milk was reduced. This amount was greater than we could account for by the contraction of the gas due to a change in temperature; neither did we desire to heat the milk lest some change might occur. That heat increases the gas liberated from a definite amount of milk is proved by Thörner's (13) work where large amounts of gas were obtained from comparatively small amounts of milk. Taking milk at $37\frac{1}{2}$ °C. or thereabouts it is believed that the milk does not undergo any change and that the greatest amount of gas could be obtained without altering the milk.

(13.) Chemiker Zeitung, 1894, Bd. XVIII.

Furthermore it was also found necessary, after the preliminary tests, to limit the time of pumping because at no time was the milk totally exhausted, although most of the gas passed off within fifteen minutes after pumping began. The time limitation was fixed arbitrarily at one hour, believing that this limitation practically precluded the possibility of fermentation.

A pressure of 756 mm. to 759 mm. was recorded upon the manometer of the Geissler pump and from these have been deducted the errors in measurement resulting from vapor tension, yielding the results recorded. With three constants, which influence the amount of gas under our control, we were able to obtain fairly uniform results. There was, however, an error creeping in occasionally due to the presence of obstructions in the upright capillary tube connecting with the container. In this respect the apparatus designed is not as perfect as it should be, for mercury would sometimes fail to be drawn over from the capillary tube extending from the container to the horizontal portion of the arm, and thus interfere with the suction power of the pump upon the milk. I have given in two or three instances the analyses of very small amounts of gas; the explanation for these small amounts will be found in such obstructions. Whenever they occurred they could easily be detected while pumping.

Pflüger in his studies has made reference to the possible atmospheric source of some oxygen and nitrogen as found in milk and places himself against such a view. Both in his work and in our analyses this relationship is not established, therefore his views are upheld. The percentage of oxygen falls far below that of the air and even below the solubility of oxygen in water. The percentages of oxygen obtained from unexposed milk are

2.88, 3.09, 2.69, 2.14, 1.6, 2.12.

These figures represent mere traces of oxygen when viewed in the light of percentage of gas volume obtained instead of milk volume, which has not been employed in the estimation of any of our percentages. The following are the percentages of oxygen in its relation to total gas less carbon dioxide; however were these figures the solubilities of these two gases, oxygen and nitrogen only, as in air, the oxygen should exist in the ratio of 33.9:66.1 to nitrogen according to Bunsen:

10.29, 14.09, 14.70, 11.86, 8.06, 15.26.

There is only one possible way of explaining the presence of oxygen in the gas-content of milk. It is barely possible that some oxygen may be accounted for by its creeping in through its adherence to the mercury and walls of the container; however, when an attempt is made to secure it in pumping in the absence of milk not an appreciable trace of gas can be obtained. This ought to demonstrate that the oxygen is inherent in the milk.

It may be also well worth considering the fact that the cow used for this purpose received very little exercise; she was confined to her stall most of the time. There may be some relation existing between the carbon dioxide and the oxygen of milk due to the amount of exercise of the animal caused by a reduction in the metabolism of the body cells. I call attention to this as a possible answer to the variation in the gas constituents of the milk.

After ascertaining the gas-content of milk before it has been exposed to the air, it became necessary, in order to fully understand the changes in the gas-content of milk, to next study the gases of milk immediately after milking.

V. ANALYSES OF THE GAS-CONTENT OF MILK DIRECTLY AFTER MILKING.

The milk was secured in the ordinary way by milking into an open receptacle, thus allowing the milk to become exposed to the air in an open stream from the milk-duct to the receptacle; further, there was the churning action taking place as the streams impinged against the surface, which also exerts a marked influence in bringing the air in contact with the milk, because by this method much air is carried down into the milk. It has been found that it takes a few minutes after milking before the perceptible air bubbles rise to the surface. The surface of the milk in the receptacle also offered an opportunity for a considerable interchange of gases between the milk and the air.

Immediately after milking the milk was taken to the laboratory and placed in the same container as in the previous analyses, and the gas was obtained in identically the same manner.

Thörner (14) has made some analyses of the gas obtained from milk immediately after milking, but he employed heat to drive off the gases. The amount of gas which he obtained was much larger than in our case and his results were usually higher in carbonic acid gas, possibly due to his methods. I take the liberty to quote several of his analyses of milk directly after milking:—

Amount of gas per L.	Per cent of CO ₂ .	Per cent of Oxygen.	Per cent of Nitrogen.	
57.0	61.6	6.4	32.0	Open to air.
85.6	73.0	5.2	21.8	Open to air.
84.5	72.8	4.4	22.8	Open to air.
68.2	70.7	4.8	24.5	Open to air.
78.6	71.7	4.7	23.6	Open to air.
64.0	65.6	7.6	27.1	Open to air.
63.0	65.0	10.0	25.0	Open to air.
57.0	55.5	11.1	33.4	Open to air.
80.0	72.8	4.4	22.8	Open to air.
68.0	70.7	4.8	24.5	Open to air.
78.6	71.7	4.7	23.6	Open to air.

In considering the work of Thörner it is necessary to have in mind the method employed by him in securing gas from milk. Inasmuch as we had reached the conclusion that a high temperature increased the amount of gas obtained from a definite quantity of milk and that probably the carbon dioxide is with difficulty released upon pumping,

(14.) Loc. cit.

issue must be taken with the results of Thörner. Further it has been demonstrated that other gases as hydrogen sulphide may be generated by boiling milk.

The high percentages of carbon dioxide indicates that heat may have facilitated the dissociation of carbon dioxide whether in solution or loose combination to that extent that a higher percentage of carbon dioxide and a lower percentage of oxygen were the results. It is well known carbon dioxide is with difficulty liberated in the presence of certain salts as CaCO_3 , Na_2HPO_4 , and others (Setchenow). At any rate as our tables will show, Thörner's average of percentages differs materially from ours, although it falls considerably below milk which has not been exposed to the air.

Blyth (15) in his analysis of the gas-content of milk immediately after milking obtained a very low amount of carbon dioxide. His analysis is,

Carbon dioxide	.06 cc.	3.27 per cent.
Nitrogen	1.42 "	77.60 " "
Oxygen	.35 "	19.13 " "

The results obtained by him in no way can be reconciled with the results of Thörner or with the results which we secured. He has shown the possibility of the development of carbon dioxide after fermentation has begun, but it appears incredible that he should have gotten such a low percentage of carbon dioxide from fresh milk.

Our analyses are as follows:—

TABLE II.

Sample.	I.	II.	III.	IV.	V.	VI.
Date.....	Nov. 29th.	Nov. 30th.	Dec. 2d.	Dec. 4th.	Dec. 6th.	Dec. 7th.
Began milking at.....	7:35 a. m.	7:25 a. m.	7:30 a. m.	7:35 a. m.	7:35 a. m.	7:45 a. m.
Completed milking at.....	7:45 a. m.	7:35 a. m.	7:40 a. m.	7:45 a. m.	7:45 a. m.	7:55 a. m.
Began pumping at.....	9:00 a. m.	8:35 a. m.	8:30 a. m.	9:00 a. m.	8:40 a. m.	8:50 a. m.
Completed pumping at.....	10:15 a. m.	9:35 a. m.	9:30 a. m.	10:15 a. m.	9:40 a. m.	9:50 a. m.
Temperature when pumping.....	37.6°	38°	38°	38°	37.8°	37.5°
Barometric pressure in mm.....	688.6	690.63	690.63	696.6	698.6	692.84
Total gas in c. cm.....	6.62	6.84	5.96	2.70	6.64	5.5
Carbon dioxide in c. cm.....	3.82	3.78	3.47	1.78	4.35	2.93
Per cent. of carbon dioxide.....	59.31	55.33	58.27	66.1	65.65	53.28
Oxygen in c. cm.....	.91	.86	.84	.32	.727	.85
Per cent. oxygen.....	13.79	12.66	14.17	11.89	10.96	15.57
Residual gas in c. cm.....	1.78	2.19	1.64	.59	1.55	1.71
Per cent. residual gas.....	26.9	32.01	27.56	22.01	23.39	31.15

(15.) Foods, Their Composition and Analysis, 4 Ed., p. 251.

In the study of the above tables it will be found that the percentage of the gases in milk have undergone a change in the milking process itself. The carbon dioxide has been reduced and the oxygen increased. The six analyses of the gas-content of milk before exposure to the air give the following percentages in carbon dioxide:

80.58, 78.35, 81.73, 81.97, 80.16 and 86.19.

The average of the six analyses is $81.49\frac{2}{3}$ per cent.

In the case of carbon dioxide immediately after milking, six analyses give in percentages

59.31, 55.33, 58.27, 66.1, 65.65 and 53.28.

The average of these is $59.63\frac{2}{3}$ per cent.

The oxygen content has also undergone a marked change by the process of milking as compared with non-exposure to the air. We obtained as the oxygen content of milk in six analyses,

2.88, 3.09, 2.69, 2.14, 1.6 and 2.12 per cent.

The average of these is 2.42 per cent.

After the milk had been exposed to the air by the process of milking there was obtained,

13.79, 12.66, 14.17, 11.89, 10.96 and 15.57.

The average of these is $13.17\frac{2}{3}$ per cent. There has therefore been an increase in the oxygen content of milk after milking over the oxygen content of unexposed milk of $10.75\frac{2}{3}$ per cent. Further the percentages of residual gas drawn from the udder without exposure to the air is

16.54, 18.56, 18.27, 15.89, 18.24 and 11.71.

The averages of these is $16.53\frac{1}{2}$ per cent. After the milking process the percentages of residual gas are in six analyses,

26.9, 32.01, 27.56, 22.01, 23.39 and 31.15.

The average of these is 27.17 per cent, a gain of $10.63\frac{1}{2}$ per cent in the residual gas over the residual gas in unexposed milk. There was a gain of oxygen and residual gas of $10.75\frac{2}{3}$ per cent and $10.63\frac{1}{2}$ per cent respectively, or in all $21.39\frac{1}{6}$ per cent. There was a loss of 21.84 per cent in the case of carbon dioxide. Therefore, we find that these averages practically offset each other.

The percentages of oxygen to total gas less carbon dioxide in milk unexposed to the air were in six analyses

10.29, 14.09, 14.70, 11.86, 8.06 and 15.26 per cent.

After milking the percentages of oxygen to total gas less carbon dioxide are in six analyses

33.83, 28.19, 33.88, 25.16, 32.16 and 31.96 per cent.

From an examination of these two sets of results assuming that we are considering the relation of oxygen to nitrogen as they exist in the air it will at once be noticed that there has been a decided interchange in the relationship of oxygen to total gas less carbon dioxide due to change of gases between the air and milk during the process of milking; further, these two sets indicate that there is a chemical change going on rather than a mere mixing of air and milk, because we should expect that were it a mere solubility of oxygen and residual gas (perhaps nitrogen) the relationship would be as 33.9 to 66.1 as has been stated.

VI. ANALYSES OF GAS-CONTENT OF MILK DIRECTLY AFTER AËRATING OVER GLASS.

To understand what is effected by aëration a study of the gas-content of milk was made after it had been subjected to a specially devised process of aëration. The common methods of aëration were not employed on account of our inability to cope with the necessary requirements of such experiments with the facilities at hand. It would be absolutely essential that in such methods, a milk should be used under our control from the time it leaves the udder, and the aëration should also have to be under our direct supervision. Since such methods were ruled out from necessity, we resorted to methods which would simulate the methods in vogue as closely as possible.

A piece of glass six feet long by two inches wide was placed on an inclined board at an angle of about twenty degrees. Over this the milk was run drop by drop, making a film approximately of the extent of surface as indicated in the table. The amount of milk aërated during the process is also given. Room temperature varying from 18° to 20°C. was employed during aëration and was found to be the only temperature which could be satisfactorily used unless a special plant for aërating were constructed. The analyses of gases obtained from milk aërated over glass were conducted the same as with milk after milking.

TABLE III.

Sample.	I.	II.	III.	IV.	V.	VI.
Date.....	Dec. 3d.	Dec. 5th.	Dec. 12th.	Jan. 3d.	Jan. 4th.	Jan. 6th.
Began milking at.....	7:25 a. m.	7:30 a. m.	8:20 a. m.	8:00 a. m.	7:50 a. m.	7:45 a. m.
Completed milking at.....	7:38 a. m.	7:40 a. m.	8:30 a. m.	8:15 a. m.	8:00 a. m.	8:00 a. m.
Began aërating at.....	7:45 a. m.	7:50 a. m.	8:40 a. m.	9:00 a. m.	8:45 a. m.	9:28 a. m.
Completed aërating at.....	8:50 a. m.	8:45 a. m.	9:40 a. m.	10:00 a. m.	9:45 a. m.	10:28 a. m.
Began pumping at.....	9:25 a. m.	9:10 a. m.	10:00 a. m.	10:30 a. m.	10:22 a. m.	11:15 a. m.
Completed pumping at.....	10:25 a. m.	10:10 a. m.	11:00 a. m.	11:30 a. m.	11:12 a. m.	12:15 p. m.
Barometric pressure in mm.....	692.632	698.632	688.632	703.772	702.632	688.632
Temperature of water in tank....	38°	38°	38°	36°	38°	38°
Total gas in c. cm.....	6.0948	4.2344	5.3384	4.1727	4.8757	6.9394
Free carbon dioxide in c. cm	2.3818	1.4418	2.0670	1.4287	2.4198	3.3107
Per cent. carbon dioxide.....	39.08	34.05	38.72	34.24	49.63	47.71
Free oxygen in c. cm.....	1.3743	.9908	1.1130	.9300	.8664	1.1561
Per cent. oxygen.....	22.55	23.4	20.85	22.29	17.77	16.66
Residual gas in c. cm.....	2.3385	1.8017	2.1583	1.8138	1.58	2.4725
Per cent. of residual gas.....	38.37	42.55	40.43	43.47	32.60	35.63
Amount of milk aërated in c. cm.	850	700	570	700	565	700
Extent of surface approximately	1½ inches.	1½ inches.	1½ inches.	1½ inches.	1½ inches.	1½ inches.

In these tables the percentages of carbon dioxide are

39.08, 34.05, 38.72, 34.24, 39.63 and 47.71.

The average of these is $40.57\frac{1}{6}$ per cent.

This represents a loss of $19.06\frac{5}{6}$ per cent of carbon dioxide by aëration over glass, for the percentages of carbon dioxide after milking were

59.31, 55.33, 58.27, 66.1, 65.65 and 53.28.

The averages of these is $59.63\frac{2}{3}$.

The percentages of oxygen obtained by aëration are

22.55, 23.4, 20.85, 22.29, 17.77, 16.66.

The average of these is $20.58\frac{2}{3}$ per cent.

In the case of milk directly after milking the percentages of oxygen were

13.79, 12.66, 14.17, 11.89, 10.96 and 15.57.

The average of these is $13.17\frac{2}{3}$ per cent.

This represents a gain of 7.41 per cent in the oxygen constituent of aërated milk over milk which has not been aërated.

The residual gas exists in the following percentages in the aërated milk,

38.37, 42.55, 40.43, 43.47, 32.60 and 35.63.

The average of these is $38.84\frac{1}{6}$ per cent.

In milk after milking,

26.9, 32.01, 27.56, 23.01, 23.39, 31.15.

The average of these is 27.17 per cent.

It thus follows that the residual gas has increased $11.67\frac{1}{6}$ per cent.

After milking the percentages of oxygen to total gas less carbon dioxide are

33.83, 28.19, 33.86, 35.16, 32.16 and 31.96.

The average of these is 32.53 per cent.

After aërating the percentages are

37.01, 35.44, 34.00, 33.89, 35.45 and 31.86.

The average of these is $34.60\frac{5}{6}$, a gain of $2.07\frac{5}{6}$ per cent by aëration.

VII. ANALYSES OF GAS-CONTENT OF MILK AFTER AËRATION OVER TIN, COPPER AND THROUGH GLASS WOOL AND COPPER SIEVES.

It was thought advisable to test different materials in place of glass inasmuch as it is well established that some metals have an influence upon oxidation. The next two tables will indicate the value of aëration over tin or copper. The last table is the result of an attempt to break up the drops of milk by passing the drops through copper sieves and through glass wool three inches thick. The drops passing in all through six feet of air before reaching the receptacle, the materials mentioned above intervening.

It was found very difficult to operate this combination of air sieves and glass wool satisfactorily so as to obtain uniform results. The trouble lay in the fact that sometimes the drops could be thoroughly shattered and at other times small streams would form.

TABLE IV. (Over tin.)

Sample.	I.	II.	III.	IV.	V.
Date.....	Dec. 20th.	Dec. 21st.	Dec. 23d.	Dec. 28th.	Jan. 7th.
Began milking at.....	7:40 a. m.	7:45 a. m.	7:45 a. m.	7:35 a. m.	7:40 a. m.
Completed milking at.....	7:50 a. m.	8:00 a. m.	8:00 a. m.	7:48 a. m.	7:50 a. m.
Began aërating at.....	7:55 a. m.	8:05 a. m.	8:05 a. m.	7:55 a. m.	8:45 a. m.
Completed aërating at.....	8:55 a. m.	9:05 a. m.	9:05 a. m.	8:55 a. m.	9:45 a. m.
Began pumping at.....	9:30 a. m.	9:30 a. m.	9:25 a. m.	9:18 a. m.	10:15 a. m.
Completed pumping at.....	10:30 a. m.	10:30 a. m.	10:25 a. m.	10:18 a. m.	11:15 a. m.
Barometric pressure in mm.....	695.265	696.632	678.632	688.672	687.242
Temperature of water in tank.....	38.5°	37.8°	37.9°	38°	37.2°
Total gas in c. cm.....	4.4822	6.4969	6.7922	6.9385	6.6669
Free carbon dioxide in c. cm.....	1.8874	1.8561	1.5900	2.4485	2.9854
Per cent. carbon dioxide.....	42.11	28.57	23.41	35.29	44.78
Free oxygen in c. cm.....	.8541	1.4715	1.7489	1.4057	1.0320
Per cent. of oxygen.....	18.61	22.65	25.75	20.26	15.48
Residual gas in c. cm.....	1.7606	3.1691	3.4531	3.0841	2.6494
Per cent. of residual gas.....	39.28	48.78	50.84	44.45	39.74
Amount of milk aërated.....	1,000 c.cm.	600 c.cm.	565 c.cm.	800 c.cm.	560 c.cm.
Extent of surface approximately.....	$\frac{3}{4}$ inch.	2 $\frac{1}{2}$ inches.	2 $\frac{1}{2}$ inches.	1 $\frac{1}{2}$ inches.	1 $\frac{1}{2}$ inches.

TABLE V. (Over copper.)

Sample.	I.	II.	III.	IV.	V.
Date.....	Dec. 10th.	Dec. 12th.	Dec. 19th.	Dec. 24th.	Dec. 26th.
Began milking at.....	7:30 a. m.	7:35 a. m.	7:50 a. m.	7:30 a. m.	7:35 a. m.
Completed milking at.....	7:40 a. m.	7:45 a. m.	8:00 a. m.	7:45 a. m.	7:50 a. m.
Began aërating at.....	7:45 a. m.	7:48 a. m.	8:10 a. m.	7:55 a. m.	8:00 a. m.
Completed aërating at.....	8:40 a. m.	9:00 a. m.	9:10 a. m.	8:55 a. m.	9:00 a. m.
Began pumping at.....	9:40 a. m.	9:30 a. m.	9:35 a. m.	9:25 a. m.	9:30 a. m.
Completed pumping at.....	10:40 a. m.	10:30 a. m.	10:35 a. m.	10:25 a. m.	10:30 a. m.
Barometric pressure in mm.....	690.632	696.937	696.937	686.632	690.632
Temperature of water in tank.....	38°	37.5°	37.6°	38°	37.8°
Total gas in c. cm.....	.6349	5.0379	3.4637	4.4236	3.4411
Free carbon dioxide in c. cm.....	.136	2.638	1.516	1.837	1.8110
Per cent. carbon dioxide.....	21.43	52.25	43.79	41.54	52.63
Free oxygen in c. cm.....	.205	.725	.588	.589	.3169
Per cent. of oxygen.....	32.34	14.41	16.99	13.33	9.21
Residual gas in c. cm.....	.293	1.679	1.358	1.99	1.3131
Per cent. of residual gas.....	46.23	33.34	39.22	45.13	38.16
Amount of milk aërated.....	1 L.	1 L.	$\frac{1}{2}$ L.	$\frac{1}{2}$ L.	1 L.
Extent of surface approximately.....	2 $\frac{1}{2}$ inches.	1 $\frac{1}{2}$ inches.	$\frac{1}{2}$ inch.	1 $\frac{1}{2}$ inches.	1 $\frac{1}{2}$ inches.

TABLE VI. (Through glass wool.)

Sample.	I.	II.	III.	IV.
Date.....	Nov. 11th.	Nov. 25th.	Nov. 26th.	Dec. 27th.
Began milking at.....	7:40 a. m.	7:35 a. m.	7:25 a. m.	7:45 a. m.
Completed milking at.....	7:50 a. m.	7:45 a. m.	7:35 a. m.	7:55 a. m.
Began aërating at.....	8:30 a. m.	8:00 a. m.	7:45 a. m.	8:00 a. m.
Completed aërating at.....	9:30 a. m.	9:00 a. m.	8:45 a. m.	9:00 a. m.
Began pumping at.....	9:45 a. m.	9:25 a. m.	9:30 a. m.	9:35 a. m.
Completed pumping at.....	10:45 a. m.	10:25 a. m.	10:30 a. m.	10:35 a. m.
Barometric pressure in mm.....	722.3932	690.632	697.265	694.632
Temperature of water in tank.....	22.8°	37.8°	38.5°	38°
Total gas in c.cm.....	4.0405	2.5399	4.9704	5.4427
Free carbon dioxide in c.cm.....	.5385	.5440	1.5810	1.9952
Per cent. of carbon dioxide.....	13.33	21.42	31.81	36.66
Free oxygen in c.cm.....	1.0771	.7711	.9940	.8844
Per cent. of oxygen.....	26.66	30.36	20.00	16.25
Residual gas in c.cm.....	2.4247	1.2247	2.3952	2.5629
Per cent. of residual gas.....	60.01	48.22	48.19	47.09
Amount of milk aërated in c.cm.....	560.	600.	750.	1.050

The studies of aëration as compared with unexposed milk and milk directly after milking can profitably be presented at this stage. The percentages of carbon dioxide are in unexposed milk

80.58, 78.35, 81.75, 81.97, 80.16, 86.19;

in milk after milking

59.31, 55.33, 58.27, 66.1, 65.65, 53.28;

in milk after aëration

over glass,

39.08, 34.05, 38.72, 34.24, 49.63, 47.61;

over tin,

42.11, 28.57, 23.41, 35.29, 44.78;

over copper,

21.43, 52.25, 43.79, 41.54, 52.63;

through glass wool and copper sieves,

13.33, 21.42, 31.81, 36.66.

The averages of these are

in unexposed milk..... 81.496 per cent.

in milk after milking..... 59.636 " "

in milk after aëration

over glass 40.572 per cent.

over tin 35.832 " "

over copper 42.328 " "

through glass wool and copper sieves 25.805 " "

In the case of oxygen the percentages are,

in an unexposed milk,

2.88, 3.09, 2.69, 2.14, 1.6, 2.12;

in milk directly after milking,
 13.79, 12.66, 14.17, 11.89, 10.96, 15.57;
 in milk after aëration
 over glass,
 22.55, 23.4, 20.85, 22.29, 17.77, 16.66;
 over tin,
 18.61, 22.65, 25.75, 20.26, 15.48;
 over copper,
 32.34, 14.41, 16.99, 13.33, 9.21;
 through glass wool and copper sieves,
 13.33, 21.42, 31.81, 36.66.

The averages of these are

in unexposed milk.....	2.42	per cent.
in milk directly after milking.....	13.176	" "
in milk after aëration		
over glass	20.586	" "
over tin	20.55	" "
over copper	17.256	" "
through glass wool and copper sieves..	25.805	" "

The residual gas has the following percentages:

in unexposed milk,
 16.34, 18.56, 18.27, 15.89, 18.24, 11.71;
 in milk after milking,
 26.9, 32.01, 27.56, 22.01, 23.39, 31.15;
 in milk after aëration
 over glass,
 38.37, 42.55, 40.43, 43.47, 32.60, 35.63;
 over tin,
 39.28, 48.78, 50.84, 44.45, 39.74;
 over copper,
 46.23, 33.34, 39.22, 45.13, 39.16;
 through glass wool and copper sieves,
 60.01, 48.22, 48.19, 47.09.

The averages of these are

in unexposed milk.....	16.535	per cent.
in milk directly after milking.....	27.17	" "
in milk after aëration		
over glass	38.842	" "
over tin	44.618	" "
over copper	40.416	" "
through glass wool and copper sieves..	50.8775	" "

The relation of oxygen to residual gas is best shown by the determination of percentages of oxygen to the residual gas plus oxygen. This is done to bring out any atmospheric relationship.

Percentages in case of

unexposed milk,
 10.29, 14.09, 14.70, 11.86, 8.06 and 15.26;
 milk after milking,
 33.83, 28.19, 33.88, 35.16 and 31.96;

milk after aëration

over glass,

37.01, 35.44, 34.00, 33.89, 35.45, 31.86;

over tin,

32.14, 31.70, 33.61, 31.30, 28.05;

over copper,

46.16, 30.15, 30.21, 22.83, 19.44;

through glass wool and copper sieves,

30.75, 38.60, 29.32, 25.65.

The average percentages are

in unexposed milk..... 12.376

in milk directly after milking..... 32.53

in milk after aëration

over glass 33.94

over tin 31.16

over copper 29.76

through glass wool and copper sieves..... 31.13

We gather from these studies that after the milk leaves the udder of the cow there is a diminution in the amount of carbon dioxide and an increase in oxygen to a certain low percentage which is dependent upon the thoroughness with which the milk is brought in contact with the air. From the extreme results it is evident there has been a falling off in carbon dioxide of 73.86 per cent and an increase of 35.06 per cent of oxygen, thus indicating that there is no equi-volumetric interchange. Even the average results show no intimate relationship between the oxygen and carbon dioxide. This may, however, be due to the difference in the solubilities of the gases, carbon dioxide being dissolved volume for volume, while oxygen enters only comparatively meagerly into solution, about four per cent by volume. It would be possible by the direct determination of carbon dioxide in milk before the beginning of fermentation to say to what extent the milk has been aërated.

Further studies of the gases of milk and their interchange will reveal other aspects of the subject.

VIII. NO APPRECIABLE INTERCHANGE OF GASES BETWEEN STERILIZED MILK AND THE AIR CONFINED OVER IT.

In six experiments an attempt was made to ascertain whether or not there was a decrease in the amount of oxygen in the air confined over sterilized milk and a development of carbon dioxide. Liter flasks were taken and 500 c. cm. of fresh milk was added and sterilized on three consecutive days for one hour each day. They were then sealed with sterilized rubber stoppers through the single perforations of which passed two way stop-cock tubes by means of which the air content could be drawn at will. Besides the rubber stopper seal, a mercury seal was made about the rubber stopper by wiring a rubber tube about the neck of the flask and allowing it to reach above the rubber stopper where it was cut off. This formed a cup shaped envelope for the stopper and could

be filled with mercury to cover the rubber stopper completely. Through the glass tube the air-content of the flask could be removed easily at will and analyzed without the possibility of extraneous air entering.

Six analyses of air from different flasks made within a period of six weeks showed no appreciable difference from that of the extraneous air used as check.

IX. *ACTION OF SPECIFIC MICRO-ORGANISMS UPON THE INTERCHANGE OF GASES BETWEEN MILK AND AIR.

It becomes necessary, after a review of the gas-content of milk when unexposed to the air, when exposed to the air by milking, and by the process of aëration, to consider what influences fermentation may have.

Of course it is well understood that micro-organisms are capable of producing different gases, yet we have felt it obligatory to make some specific studies in this connection. Consequently several micro-organisms isolated from milk and known by their laboratory numbers were used to inoculate sterilized milk and the flasks sealed as described under V.

* See description of bacteria on page 35.

TABLE VII.
STUDY OF INTERCHANGE OF GASES CONTAINED OVER MILK INOCULATED WITH SPECIFIC MICRO-ORGANISMS.

Culture of Micro-Organisms.	No. 119.	No. 120.	No. 121.	No. 122.	No. 123.	No. 124.	No. 125.	No. 126.	No. 128.	No. 129.	No. 130.
Date of sample.....	Aug. 28	Aug. 28	Aug. 28	Aug. 28	Aug. 28	Aug. 28	Aug. 25	Aug. 28	Aug. 28	Aug. 28	Aug. 28
Date of analysis.....	Sept. 23	Sept. 24	Sept. 23	Sept. 26	Oct. 10	Sept. 28	Oct. 2	Oct. 18	Oct. 10	Oct. 15	Oct. 23
Amount of milk used in c.cm.....	100	100	100	100	100	100	100	100	100	100	100
Amount of air space over milk in c.cm.....	225	217	220	220	216	221	179	213	225	213	218
Amount of gas examined in c.cm	35.8	27.0	26.0	26.8	21.85	28.2	23.6	30.65	31.15	21.35	19.00
Amount of carbon dioxide in c.cm	3.4	16.0	0	15.4	1.25	12.8	2.2	.0	7.45	.1	2.2
Per cent. of carbon dioxide.....	9.49	59.25	0	57.46	5.72	45.39	9.32	.0	23.91	.47	11.052
Amount of oxygen in c.cm.....	1.0	0	4.6	0	0	0	0	1.50	.2	.05	1.2
Per cent. of oxygen.....	2.79	0	17.69	0	0	0	0	.42	.6	.20	6.315
Amount of residual gas in c.cm...	31.4	11.0	21.4	11.4	26.60	15.4	21.4	29.35	23.5	21.20	15.6
Per cent. of residual gas.....	87.72	40.75	82.31	42.54	94.28	54.61	90.63	99.995	76.08	99.33	82.633

It will at once be recognized that no attempt has been made to determine any other gases which may be present than carbon dioxide and oxygen because with these alone are we mostly concerned. Hydrogen and marsh gas were determined in several instances and the results obtained were included with the total residual gas.

In most analyses, it is worthy of emphasis, there have been noticeable decreases in the amount of oxygen and increases in carbon dioxide. This, however, is not universally true because in No. 121 there has been no production of carbon dioxide and the oxygen has been but little reduced. In No. 126 there are no traces of carbon dioxide and still the oxygen has been practically consumed. In short there is a wide diversity of action, each micro-organism seems to be governed by its own peculiar functions. As a rule, however, there seems to be sufficient proof that oxygen dioxide is a common product of these micro-organisms and that oxygen is consumed to a greater or less degree.

X. THE INTERCHANGE OF GASES BETWEEN MILK FRESH FROM THE COW AND CONFINED AIR.

To ascertain whether changes indicated above took place fresh milk was taken and flaked as in the previous experiments and after a time the confined air analyzed.

However, before detailing the results of these experiments it may be desirable to quote the work of Dr. Felix Hoppe ("Arch. f. Path. Anat. u. Physiol. u. f. klinische Medicin" Bd. 17, S. 417, (1859) whose results are as follows:—

Sample.	Vol. of air.	Vol. of milk.	Per cent of CO ² .	Per cent of oxygen.	Per cent of nitrogen.	Hours confined together.
I.....	588	500	6.08	13.03	80.89	24
II.....	228	100	13.30	8.40	78.30	24
III.....	178	150	15.99	8.39	75.62	24
IV.....	185	140	10.94	12.64	76.42	24
V.....	156	140	35.95	0	64.05	96
VI.....	175	150	33.05	0	66.95	96
VII.....	289	185	28.58	3.76	67.66	96

E. Mathieu and D. Urbain (Compt. rend. 75, 1482) have the following experiments which indicate an interchange of carbon dioxide and oxygen when air is confined over milk :

10 c. cm. of milk was employed
at 10° C.

	In 2 hours.	In 18 hours.	In 48 hours.	In 3 days.	In 8 days.
Oxygen absorbed in c.cm.....	0.9	1.32	1.75	2.46	5.66
Carbon dioxide giving off c.cm.....	traces.	0.40	1.2	2.20	6.00

	At 18° C.	At 32° C.
	In 24 hours.	In 24 hours.
Oxgen absorbed in c.cm.....	2.62	5.00
Carbon dioxide given off in c.cm.....	3.21	5.82

Our results are as follows :—

TABLE VIII.

(Sample A.)

Amount of milk confined=1040 c. cm.
Amount of air space over milk=520 c. cm.
Temperature at which sample was maintained=37½° C.

	After 16 hours.		After 40 hours.		After 88 hours.	
Amount of gas ana- lyzed	29.8 c.cm.		26.4 c.cm.		48.5 c.cm.	
	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.
Carbon dioxide.....	1.8	6.04	3.6	13.63	10.1	20.83
Oxygen.....	4.6	15.47	1.0	3.79	0.0	0.0
Residual gas.....	22.0	78.49	21.8	82.58	38.4	79.17

(Sample B.)

Amount of milk confined=985 c. cm.

Amount of air space over milk=747 c. cm.

Temperature at which sample was maintained= $37\frac{1}{2}^{\circ}$ C.

	After 16 hours.		After 45 hours.		After 112 hours.	
Amount of gas analyzed	37.2 c.cm.		35.6 c.cm.		38.68 c.cm.	
	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.
Carbon dioxide.....	1.2	3.84	7.6	21.35	17.4	55.08
Oxygen.....	5.4	17.31	1.2	3.37	0.0	0.0
Residual gas.....	24.6	78.85	26.8	75.28	21.2	44.92

(Sample C.)

Amount of milk confined=905 c. cm.

Amount of air space over milk=750 c. cm.

Temperature at which sample was maintained= 21° C.

	After $18\frac{1}{2}$ hours.		After $42\frac{1}{2}$ hours.		After 91 hours.	
Amount of gas analyzed	33.0 c.cm.		32.6 c.cm.		32.8 c.cm.	
	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.
Carbon dioxide.....	.5	1.51	1.4	4.29	6.4	19.51
Oxygen	6.6	20.0	5.6	17.18	.2	.6
Residual gas.....	25.9	78.49	25.6	78.53	26.2	79.89

(Sample D.)

Amount of milk confined=805 c. cm.

Amount of air space over milk=770 c. cm.

Temperature at which sample was maintained=10° C.

	After 22 hours.		After 47 hours.		After 118 hours.	
Amount of gas analyzed.....	30.2 c.cm.		24.4 c.cm.		9.4 c.cm.	
	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.
Carbon dioxide.....	.2	.66	.2	.8	2.	2.13
Oxygen.....	6.2	20.5	5.0	20.49	1.6	17.01
Residual gas.....	23.8	78.8	19.2	78.71	7.6	80.86

(Sample E.)

Amount of milk confined=835 c. cm.

Amount of hydrogen over the milk=915 c. cm.

Temperature at which sample was maintained=37½° C.

	After 19 hours.		After 39½ hours.		After 114 hours.	
Amount of gas analyzed.....	32.0 c.cm.		33.6 c.cm.		37.2 c.cm.	
	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.
Carbon dioxide.....	.6	1.88	2.4	6.55	25.2	67.71
Oxygen.....	0.0	0.0	0.0	0.0	0.0	0.0
Residual gas.....	31.4	98.12	31.2	93.45	12.	32.29

(Sample F.)

Amount of milk confined=1240 c. cm.

Amount of hydrogen over milk=475 c. cm.

Temperature at which sample was maintained=21° C.

	After 21 hours.		After 42½ hours.		After 94 hours.	
Amount of gas analyzed	29.4 c.cm.		33.2 c.cm.		42.0 c.cm.	
	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.	Amount of gas obtained in c.cm.	Per cent. of gas by vol.
Carbon dioxide.....	.2	.69	.5	1.5	2.8	6.66
Oxygen.....	.2	.69	.5	1.5	0.0	0.0
Residual gas.....	29.0	98.62	32.2	97.00	39.2	93.34

These tables confirm the work with specific micro-organisms and demonstrate that there is no uniformity in the results obtained on studying the interchange of gases due to the fermentations induced by bacteria. Usually oxygen entirely disappears and carbon dioxide increases in amount, still it is found that traces of oxygen remain in some cases of air confinement over milk even after many hours. Unless there is induced fermentation, there can be no elimination of carbon dioxide and decrease of oxygen. Further, after milk reaches an age where bacterial growth is abundant there can be no correct study of the gas-content of milk.

Thörner and Blyth have further demonstrated that as fermentation sets in the carbon dioxide increases and oxygen may totally disappear.

It is apparent therefore that micro-organisms enter intimately into the production of carbon dioxide and the absorption of oxygen after fermentation begins. This may be checked by the use of antiseptics in milk.

XI. ACTION OF ANTISEPTICS UPON THE INTERCHANGE OF GASES BETWEEN MILK AND AIR CONFINED OVER IT.

Attempts were made employing chloroform, ether, formaldehyde, but without success because of their interference with the gas analyses.

Mercuric chloride and salicylic acid were also used but they were not satisfactory.

Lysol gave us the most trustworthy results.

TABLE IX.

ACTION OF LYSOL UPON GASEOUS INTERCHANGE IN MILK.

Sample.	A.		B.		C.		D.		E.		F.	
Time before analysis.....	90 hours.		13 days.		20 days.		28 days.		29 days.		90 hours.	
Per cent. of lysol used.....	1		2		1.		1.		2.		Control.	
Temperature of sample.....	37½		Room.		Room.		Room.		Room.		37½	
Amount of gas analyzed.....	30.6 c.cm.		17.4 c.cm.		28.6 c.cm.		31.6 c.cm.		45.4 c.cm.		37.0 c.cm.	
	Amount of gas obtained in c.cm. Per cent. of gas per volume.		Amount of gas obtained in c.cm. Per cent. of gas per volume.		Amount of gas obtained in c.cm. Per cent. of gas per volume.		Amount of gas obtained in c.cm. Per cent. of gas per volume.		Amount of gas obtained in c.cm. Per cent. of gas per volume.		Amount of gas obtained in c.cm. Per cent. of gas per volume.	
Carbon dioxide.....	.4	1.31	.2	1.15	.2	.7	0	0	0	0	6.2	22.97
Oxygen.....	4.6	15.03	3.0	17.44	5.0	17.51	4.0	12.66	5.6	12.33	0	0
Residual gas.....	25.6	83.66	14.2	81.41	23.4	82.42	27.6	87.34	39.3	87.67	20.8	77.03
Amount of milk confined....	1,000 c.cm.		250 c.cm.		500 c.cm.		500 c.cm.		250 c.cm.		1,000 c.cm.	
Amount of air space over sample.....	720 c.cm.		310 c.cm.		605 c.cm.		625 c.cm.		265 c.cm.		725 c.cm.	

From this we gather that some oxygen has been absorbed as is indicated by the lower percentage. It may be, however, that the carbon dioxide, had any been evolved, was absorbed by the alkalinity of the lysol. This element of error may be overcome by the use of trikresol, a neutral substance, which yielded these results:—

Five hundred c. cm. of fresh milk was placed in a liter flask to which was added one per cent. of trikresol. The flask was then mercury sealed and placed away for twenty-five days. An analysis made of the gases confined over the milk resulted as follows:

Total amount of gas analyzed.	19.6 c. cm.		
Carbon dioxide.....	0 c. cm.	0	per cent.
Oxygen	3.6 c. cm.	18.37	" "
Residual gas	16.0 c. cm.	81.63	" "

Here again the oxygen is gradually diminishing but no carbon dioxide appears to take its place as is the case with fermentation. The reason given for the failure of carbon dioxide to appear in the case of lysol will not hold with the neutral trikresol.

Milk to which lysol or trikresol has been added cannot be regarded as normal milk, yet from this work there is indicated an absorption of

oxygen; carbon dioxide is not found present, but should normal conditions be maintained there is a possibility of its appearance. Furthermore what carbon dioxide exists in the milk as gas could with difficulty be detected by an analysis of the confined air over the milk.

XII. THE ACTION OF 100 PER CENT. OF CARBON DIOXIDE UPON THE DEVELOPMENT OF BACTERIA.

From the results of the foregoing work with the gas-content of milk we are now led to a study of the influence of carbon dioxide upon micro-organisms, for it is a general and an indefinite belief that aëration has a direct bearing upon the bacterial life usually present in milk. Inasmuch as there is a high percentage of carbon dioxide in milk, it is probable that its presence might have some effect upon the development and increase of the bacteria found and thus change the course of possible milk fermentations. That carbon dioxide does exert an influence has been shown by Delbrück and Foth (16), Hansen (17), Ortloff (18). The gist of the conclusions reached by the experimental labors of these men, although not wholly in unison, is found in the restraining effect which carbon dioxide has upon the multiplying power of the yeast cell and upon its fermenting energy; also in its stimulating action upon the individual fermenting capacity of the yeast cell apparently in its search for an oxygen supply. For outward manifestations, there would appear only a restraint in fermentation development. To arrive at a comprehensive understanding of the situation in its most direct relation, carbon dioxide under varying conditions was employed to definitely ascertain its influence upon micro-organisms. In the first trial carbon dioxide in its free state was used to determine its direct bearing upon micro-organisms in plate cultures.

To accomplish this, two sets of plates as nearly identical as possible were made of the same micro-organisms; one set to be kept in the air and the other to be confined in Novy's anaërobic plating apparatus into which carbon dioxide was passed until the percentage reached one hundred. These plates were allowed to develop 48 hours or until they were thought to be fully developed, when the plates from the anaërobic apparatus were removed and both sets counted at the same time and closely compared. In all of this work pains-taking care was exercised to observe the most careful technique in securing exact conditions in every respect for the two sets of plate cultures studied—a single batch of media, the same diluted fresh bouillon culture, the same loop for measurement, the same environment during development excepting the factor, carbon dioxide,—all were brought under the closest scrutiny. The carbon dioxide used was generated from sodium carbonate and sulphuric acid. The results of these studies are tabulated below:—

(16.) *Wochenschrift für Brauerei*, 1887, p. 37.

(17.) *Cent. f. Bakt.* Bd. I, 1887.

(18.) *Cent. f. Bakt.* Bd. VI, S. 676, 721, 753.

TABLE X.

No. of micro-organism.	Plates developed in 100% CO ₂ .		Plates developed in air as check.	
	Growth.	No. of colonies.	Growth.	No. of colonies.
119.....				
120.....	† r. g.	4,400	* v. g.	4,570
121.....	‡ n. g.		v. g.	1,399
122.....	r. g.	7,549	v. g.	7,441
123.....	n. g.		v. g.	1,727
124.....	r. g.	525	v. g.	636
125.....	n. g.		v. g.	119
126.....	n. g.		v. g.	381
127.....	n. g.		v. g.	560
128.....	r. g.	3,650	v. g.	3,720
129.....				
130.....	n. g.		v. g.	1,236
131.....	r. g.	3,434	v. g.	10,303
132.....				
133.....				
134.....				

* v. g.—Vigorous normal growth of colonies.

† r. g.—Retarded growth of colonies.

‡ n. g.—No growth of colonies.

From the above table it will be readily noticed that 100 per cent of carbon dioxide has not only a marked retarding influence, but in some instances an inhibitory action where the germs have not grown at all. The retarding action was noticeable in the slow development of the colonies in the plates subjected to carbon dioxide, when in air duplicate colonies developed very rapidly. This restraining action is manifest in five out of eleven. The inhibitory action was determined by allowing the plates to develop in air after removal from the carbon dioxide atmosphere. This inhibitory action existed in six out of eleven. Total inhibition existed in Nos. 123, 125, 127. To what extent the amount of carbon dioxide may exercise any influence is of considerable interest, since we find a great variation in the carbon dioxide in milk as it undergoes the various operations of the dairy; consequently we have carried the work a little farther to ascertain the effect as the percentage is reduced.

XIII. DESCRIPTION OF MICRO-ORGANISMS.

The laboratory numbers used to represent the micro-organisms may be elucidated by reference to a table giving some identifying marks, although in the case of those bacteria obtained from milk directly, no attempt has been made to work out their life histories. They are in every case micro-organisms which have been found more or less constantly in the college dairy, have been used frequently in our laboratory work because of their frequency in the college dairy milk and are therefore representative of the class of "milk bacteria." A brief descriptive sentence is attached to each micro-organism to indicate in particular its action upon milk.

Our purpose in selecting a representative group of this nature for close study, was to gain not only information regarding the individual response of these germs to the studies undertaken, but also the results ensemble. An attempt was made to take ordinary market milk for these studies, but this after several trials was given up, because it gave no analytical significance in this application.

TABLE XI.

No. of micro-organism.	Description.
119.....	Orange pigment producing bacillus capable of peptonizing milk rapidly, after first curdling the milk.
120.....	Sours milk rapidly, curdling it and after standing for a long period slightly peptonizes it. It is a short bacillus. Gas is produced in abundance.
121.....	A short bacillus. No gas is produced. Gives rise to solid curd and large amount of acid, but does not cause peptonization of the milk.
122.....	Similar to No. 120. In some respects it differs but in general belongs to same type.
123.....	A long bacillus which peptonizes milk very rapidly. Curd formed before peptonization.
124.....	Gassy cheese germ described in Bulletin 183.
125.....	A long bacillus peptonizing milk very rapidly but different from 123 in many respects.
126.....	A coccoid bacillus producing no apparent change in milk.
127.....	A medium sized bacillus which dissolves casein rapidly without producing any distinct curd.
128.....	Of same type as numbers 120 and 122 but differing in details.
129.....	Hay bacillus—very weak. Stock culture.
130.....	Potato bacillus. Stock culture.
131.....	Colon bacillus. Stock culture.
132.....	Eberth bacillus. Stock culture.
133.....	Ropy cream bacillus as described in bulletin 140 of this station.
134.....	Hueppe's bacillus acidi latici. Stock culture.

XIV. THE ACTION OF 96.5 PER CENT. OF CARBON DIOXIDE UPON THE DEVELOPMENT OF BACTERIA.

Table XII represents culture plates made in exactly the same way and allowed to develop under identically the same conditions as in Table X, with this exception, the carbon dioxide in the aërating apparatus was reduced to 96.5 per cent.

TABLE XII.

No. of micro-organism.	Plates developed in 96.5% CO ₂ .		Plates developed in air as check.	
	Growth.	No. of colonies.	Growth.	No. of colonies.
119.....	n. g.		v. g.	3,480
120.....	r. g.	2,040	v. g.	2,000
121.....	n. g.		v. g.	1,280
122.....	r. g.	2,640	v. g.	2,060
123.....	n. g.		v. g.	1,920
124.....	r. g.	1,650	v. g.	1,900
125.....				
126.....	n. g.		v. g.	260
127.....	n. g.		v. g.	270
128.....	r. g.	3,000	v. g.	2,800
129.....				
130.....	r. g.	200	v. g.	660
131.....	r. g.	1,200	v. g.	1,870
132.....	n. g.		v. g.	3,600
133.....	n. g.		v. g.	2,060
134.....	n. g.		v. g.	4,400

Again the retardation and inhibition of the growth of micro-organisms appear to be about the same as in Table X. No development occurred in 119, 121, 123, 126, 127, 132, 133, 134 or eight out of sixteen and 119, 123, 125, 127, 133 were wholly inhibited or even destroyed for they did not develop after exposure to air. The restraining influence therefore was exerted in every instance, extending to total inhibition or destruction in the cases cited.

XV. THE ACTION OF 62.9 PER CENT. OF CARBON DIOXIDE UPON THE DEVELOPMENT OF BACTERIA.

Carrying this investigation still further we find in Table XIII the conditions identical with the previous tables but the carbon dioxide has been reduced to 62.97 per cent.

TABLE XIII.

No. of micro-organism.	Plates developed in 62.97% CO ₂ .		Plates developed in air as check.	
	Growth.	No. of colonies.	Growth.	No. of colonies.
119.....	n. g.		v. g.	5,434
120.....	r. g.	3,408	v. g.	3,280
121.....	r. g.	2,968	v. g.	4,346
122.....	r. g.	6,360	v. g.	6,140
123.....	n. g.		v. g.	3,230
124.....	r. g.	2,380	v. g.	2,475
125.....				
126.....	n. g.		v. g.	960
127.....	n. g.		v. g.	1,720
128.....	r. g.	3,234	v. g.	3,432
129.....				
130.....	r. g.	200	v. g.	953
131.....	r. g.	1,716	v. g.	2,108
132.....	n. g.		v. g.	7,000
133.....	n. g.		v. g.	4,865
134.....	n. g.		v. g.	2,520

Under the influence of 62.97 per cent of carbon dioxide we begin to find that upon some of the micro-organisms, as in the case of 121, the influence of carbon dioxide is not so marked as in the previous tables; and when, as in the case of Table XIV, the carbon dioxide has been reduced to 32.9 per cent, the restraining influence is but slight with most of the micro-organisms studied.

XVI. THE ACTION OF 32.9 PER CENT. OF CARBON DIOXIDE UPON THE DEVELOPMENT OF BACTERIA.

TABLE XIV.

No. of micro-organism.	Plates developed in 32.9% CO ₂ .		Plates developed in air as check.	
	Growth.	No. of colonies.	Growth.	No. of colonies.
119.....	n. g.		v. g.	5,200
120.....	v. g.	2,880	v. g.	3,020
121.....	v. g.	402	v. g.	510
122.....	v. g.	1,540	v. g.	1,960
123.....	r. g.	2,060	v. g.	4,240
124.....	v. g.	900	v. g.	1,000
125.....				
126.....	n. g.		v. g.	3,160
127.....	n. g.		v. g.	900
128.....	v. g.	412	v. g.	502
129.....				
130.....	r. g.	1,420	v. g.	480
131.....	v. g.	1,800	v. g.	1,720
132.....	n. g.		v. g.	1,800
133.....	n. g.		v. g.	1,520
134.....	r. g.	560	v. g.	980

v. g. of CO₂ column is not so fully developed as v. g. in air and might be considered more correctly a good growth.

In most cases there is a vigorous growth which is noted in the table, yet this vigorous growth is not so far advanced as that in the air plates. The action of carbon dioxide is slight as compared with the preceding tables. While four out of fifteen have failed to develop, still six of the previously retarded have developed vigorously. Nos. 123 and 130 which have been so susceptible to the action of free carbon dioxide now present colonies of slow development only. The conclusion therefore to be drawn from this table is the amount of carbon dioxide used approximates the border line between carbon dioxide as it exists in an active form and where it begins to manifest little influence upon the development of bacteria.

It is noteworthy that the percentages of carbon dioxide which just inhibits stands so near to the percentages of carbon dioxide obtained from milk after the aerating methods have been employed as described in the foregoing pages. We are led to believe that some relationship exists between the carbon dioxide content of milk and the rate of fermentations evolved through the agency of bacteria. This conclusion will be more strongly enforced in the tables which follow.

XVII. THE ACTION OF CARBON DIOXIDE UPON BACTERIA WHEN CULTIVATED IN MILK.

To simulate the carbon dioxide content of milk, it became necessary to employ sterilized milk tubes into which were inoculated the various micro-organisms under consideration and to subject one set of these tubes to the action of carbon dioxide conditions. For this purpose we inoculated with identical amounts of the various germs two sets of milk tubes, one set remained exposed to the air, the other set was placed in Novy's anaërobic bottle into which was passed 77.7 per cent. of carbon dioxide. This was allowed to stand twenty hours when plates were made from the aërobic set and at the same time from the anaërobic set. Care was taken to use the same measure for each germ under study. In the same way the second series was executed, but instead of using 77.7 per cent of carbon dioxide, 60.02 per cent was used. The result of this work will be found in the table below:—

TABLE XV.

ACTION OF CARBON DIOXIDE UPON BACTERIA WHEN CULTIVATED IN MILK.

No. of micro organism.	I.		II.	
	No. of colonies in one measure from		No. of colonies in one measure from	
	Tube milk cultures grown in 77.7% CO ₂ .	Tube milk cultures grown in air as check.	Tube milk cultures grown in 60.02% CO ₂ .	Tube milk cultures grown in air as check.
119.....	256	10868	6460	34660
120.....	64484	2920320	28620	71550
121.....	34320	25430	30719	24708
122.....	114400	572000	25758	57240
123.....	10863	71500	968	7200
124.....	68640	143100	19461	33580
125.....	6840	22880	620	20460
126.....	1716	34320	193	3444
127.....	9152	102966		
128.....	172600	257400	18889	21083
129.....	25	1600		
130.....			1621	2671
131.....	21736	286000	17588	17935
132.....	2432	21736	200	5600
133.....	2860	6292	4160	35300
134.....	5720	64074	580	8160

Sterilized milk from which the carbon dioxide has been expelled may be inoculated with micro-organisms, and if exposed to the action of an atmosphere of carbon dioxide shows some retardation in the development of these micro-organisms. Furthermore, the action of the carbon dioxide upon milk cultures corresponds very closely to the action of carbon dioxide upon plate cultures. There appears to be the same retarding and, in some cases, inhibiting influences at work as had been so noticeable in the preceding tables.

XVIII. ACTION OF CARBON DIOXIDE UPON BACTERIA WHEN CULTIVATED IN BOUILLON.

The results given in the following table were obtained by taking bouillon tubes and inoculating two sets; one set was placed in the air as check and the other was placed in 88.5 per cent. carbon dioxide. The methods of measurement employed were identical with those in the previous milk culture experiment.

TABLE XVI.

ACTION OF CARBON DIOXIDE UPON BACTERIA WHEN GROWN IN BOUILLON.

No. of micro-organism.	Tubes grown in 88.5% CO ₂ .	Tubes in air as check.
119.....	No growth.....	Dense growth.
120.....	*Retarded development...	Dense growth.
121.....	No growth.....	Slight development.
122.....	Retarded development....	Dense growth.
123.....	No growth.....	Dense growth.
124.....	Retarded development....	Dense growth.
125.....	No growth.....	Dense growth.
126.....	No growth.....	Dense growth.
127.....	No growth.....	Dense growth.
128.....	Retarded development....	Dense growth.
129.....	No growth.....	Dense growth.
130.....	†Slight development.....	Dense growth.
131.....	Retarded development....	Dense growth.
132.....	Slight development.....	Dense growth.
133.....	No growth.....	Dense growth.
134.....	Slight development.....	Dense growth.

* Retarded development. Slightly less cloudy than air tubes.

† Slight development. Growth only perceptible.

Table XVI clearly demonstrates also that the density of development of the two sets of bouillon tubes varies, and that in some cases there is no development so far as cloudiness is any indication, while in other cases there has only been a retarding action. The results in this table agree with those obtained in plate and milk cultures. (Tables X, XII, XIII, XIV, XV.)

XIX. HOW AN ATMOSPHERE OF CARBON DIOXIDE INFLUENCES THE CHARACTER OF THE FERMENTATION OF MILK.

To pursue a study of this subject we have felt it necessary to use the so called Hesse flasks. These flasks were filled with milk which after sterilizing were inoculated. The air in the flasks above the milk was expelled by passing carbon dioxide until complete substitution had been made. Since as shown in the previous tables the action of carbon dioxide is marked by its retarding or inhibiting the growth of bacteria it is not necessary to consider it further but rather to study the peculiar influence which the gas exerts upon the character of the fermentation of milk caused by specific bacteria.

TABLE XVII.

HOW AN ATMOSPHERE OF CARBON DIOXIDE INFLUENCES MILK FERMENTATIONS.

*No. of micro-organism.	119.	120.
Culture in air.....	Complete peptonization with yellow pigment.	Soft curd.
Culture in CO ₂	Curdled and no pigment, perhaps a very slight peptonization.	No change apparent.

No. of micro-organism.	121.	122.	123.	125.
Culture in air.....	Solid curd.	Solid curd.	Peptonized completely.	Peptonized completely.
Culture in CO ₂	Solid curd.	No apparent change.	Curdled only.	Curdled only.

No. of micro-organism.	126.	128.	129.	130.
Culture in air	No apparent change.	Curdled.	Peptonized completely.	Peptonized completely.
Culture in CO ₂	No apparent change.	Curdled.	Curdled.	No apparent change.

No. of micro-organism.	131.	132.	133.	134.
Culture in air.....	Solid curd.	No apparent change.	No change.	Solid curd.
Culture in CO ₂	No change perceptible.	No apparent change.	No change.	No change.

*These cultures were one month old before recording results.

The general conclusion to be reached from a study of this table is the checking or total inhibition of the fermentation. In germ 119, a pigment micro-organism, the pigment has entirely failed to develop. In other cases the air check has given rise to a slight curd, whereas in the carbon dioxide flask there was no apparent change in the milk. The peptonizing germs which are capable of completely peptonizing milk when exposed to air, in the carbon dioxide flask, apparently stopped with the curdling of milk and only very slight if any peptonization. It follows therefore, that carbon dioxide may influence the character of the fermentation even when allowed to continue for one month. These differences begin to appear within twenty-four hours after inoculation and persist throughout. Yet where complete peptonization occurs in the air cultures and only curdling in the carbon dioxide cultures, it must be understood that the curdling is also an intermediate stage in the fermentation of the air cultures. At this point I desire to call the reader's attention to the comparison of Table (No. XXIV) which indicates the retarding influences or inhibiting influences of unaërated milk upon bacteria. These will be found in later discussions.

XX. ACTION OF HYDROGEN UPON THE DEVELOPMENT OF SPECIFIC MICRO-ORGANISMS.

It is pertinent to place against the previous work, the action of hydrogen upon the micro-organisms which have been studied thus far. The purpose of this is, not only to check the previous work but to indicate whether the results obtained thus far are entirely due to carbon dioxide present, or to what may be regarded as the anaërobic conditions. To speak of these conditions without defining exactly their nature must, of course, leave us somewhat in darkness, but this must always remain so until more is known concerning anaërobic life. It is a common practice to use hydrogen in securing anaërobic conditions; for this reason, a comparison of hydrogen with carbon dioxide may be of interest. The methods were the same as in the previous work. Table XVIII tabulates the results.

TABLE XVIII.

ACTION OF HYDROGEN UPON THE DEVELOPMENT OF SPECIFIC MICRO-ORGANISMS.

No. of micro-organism.	Plates developed in hydrogen.		Plates developed in air as check.	
	Growth.	No. of colonies.	Growth.	No. of colonies.
119.....	n. g.		v. g.	1,200
120.....	r. g.	1,500	v. g.	1,540
121.....	v. g.	4,592	v. g.	5,166
122.....	r. g.	3,200	v. g.	4,480
123.....	n. g.		v. g.	1,158
124.....	r. g.	6,400	v. g.	9,600
126.....	n. g.		v. g.	3,100
127.....	n. g.		v. g.	720
128.....	r. g.	3,900	v. g.	4,576
129.....				
130.....	r. g.	1,500	v. g.	4,400
131.....	r. g.	1,300	v. g.	1,580
132.....	n. g.		v. g.	1,120
133.....	n. g.		v. g.	1,440
134.....	v. g.	1,680	v. g.	1,740

From this table there will be found a very close similarity in the results as found in the case of carbon dioxide. The differences are so slight that in a general conclusion they may be overlooked.

XXI. ACTION OF NITROGEN UPON THE SPECIFIC MICRO-ORGANISMS STUDIED.

Nitrogen in this case is considered, or the residual gas left after the absorption of oxygen from the air by the use of alkaline pyrogallate. Of the plate cultures, one set remained in the air as check and the other set was placed in a jar from which the oxygen was absorbed. The plates were made by the same methods used heretofore. The results are tabulated in Table XIX.

TABLE XIX.

No. of micro-organism.	Plates developed in nitrogen. (air-oxygen).		Plates developed in air as check.	
	Growth.	No. of colonies.	Growth.	No. of colonies.
119.....	v. g.	8,320	v. g.	9,600
120.....	v. g.	9,000	v. g.	8,400
121.....				
122.....	v. g.	1,850	v. g.	1,800
123.....	r. g.	1,625	v. g.	1,785
124.....	v. g.	2,200	v. g.	2,324
126.....	r. g.	256	v. g.	3,840
127.....	n. g.		v. g.	1,508
128.....				
129.....				
130.....	Liquefied.		Liquefied.	
131.....	v. g.	2,020	v. g.	1,785
132.....	r. g.	5,120	v. g.	5,760
133.....	r. g.	880	v. g.	1,560
134.....	v. g.	innum'ble.	v. g.	innum'ble.

Nitrogen or the residual gas resulting from the absorption of oxygen from the air does not appear to have the same detrimental effect upon these micro-organisms as either the carbon dioxide or hydrogen. We are led to believe, therefore, that the gases themselves play an important part in the restraining influences exercised when these gases are employed and the injurious effects observed may be counted as reaching beyond that which may be considered as purely anaërobic conditions. There is a retarding and inhibiting influence caused by carbon dioxide and also by hydrogen. In the case of milk, it must of course be due to carbon dioxide alone so far as the action of a gas is concerned. It is generally considered that hydrogen is not an inert gas when employed for the cultivation of anaërobic bacteria (Novy-Cent. f. Bakt. XIV 586). Liborius has shown that carbon dioxide likewise is not suitable as an atmosphere for anaërobiosis (Cent. f. Bakt. III-768). Fraenkel in the case of symptomatic anthrax bacillus and malignant edema bacillus

found carbon dioxide completely checking the growth and apparently killing the bacteria (Zeit. f. Hyg. V-323).

The conclusion reached regarding nitrogen should be regarded as indicative of a wide influence exerted by gases in the production of anaërobiosis. While the prime essential to anaërobiosis is an absence of oxygen, yet there must usually be associated with this absence a substitute gas which necessarily imparts to the germ environment favorable or unfavorable factors in the determination of active cell metabolism and cell growth. It becomes therefore exceedingly difficult to definitely state the actual conditions of anaërobiosis.

Before arriving at a definite and specific notion of what is involved in the process of aëration, other factors, other than carbon dioxide have to be considered. One of these is the relation of acidity to the growth and increase of micro-organisms.

XXII. THE INFLUENCE OF LACTIC ACID AS FOUND IN WHEY OBTAINED FROM MILK OF DIFFERENT DEGREES OF ACIDITY.

It is necessary after studying the influence of carbon dioxide upon the growth of micro-organisms in milk and ascertaining its probable influence and to what extent its influence may be exerted, to consider the action of lactic acid upon germ life as it is produced during the fermentation of milk. Moreover no factor which apparently has a direct bearing upon the germ-content of milk can be eliminated without a possible detriment to the conclusions about to be drawn later, consequently it is evident that this item has a very pertinent connection. It is well established that anaërobic conditions sometimes alter the acidity of cultures, primarily as the result of the functional activity of the cell influenced by the exclusion of oxygen. The important bearing of this fact will be brought out more prominently as we continue our studies.

We have sought to obtain the actual fermentation products without materially interfering with the food qualities of the milk. It is well known that as soon as the acidity increases slightly, the milk is more likely to curdle during the process of sterilization; in other words, heat favors the curdling of milk in the presence of traces of acid while the same milk in the cold shows no formation of curd. It is evident that in order to secure the fermentation products, the curd must be removed and some other nitrogenous substance added to take its place. The milk was allowed to sour for variable lengths of time after which it was heated. The curd was now removed and one per cent. of peptone added. The media were then clarified and the acidity determined when they were sterilized in the ordinary manner. The media thus prepared and having different acidities were inoculated with the various micro-organisms. In this way the influence of the fermentation products could be studied.

The table below indicates by a (+) plus sign the micro-organisms having grown and the (—) minus sign the micro-organisms having failed to grow.

TABLE XX.

THE INFLUENCE OF LACTIC ACID AS FOUND IN WHEY OBTAINED FROM MILK OF DIFFERENT DEGREES OF ACIDITY.

Per cent acidity (estimated as lactic acid)	.162	.261	.306	.333	.351
No. of micro-organism.					
119.....	+	—	—	—	—
120.....	+	+	+	+	—
121.....	+	—	—	—	—
122	+	+	+	+	—
123.....	+	+	+	+	—
124.....	+	+	—	—	—
125.....	+	—	—	—	—
126.....	+	—	—	—	—
127.....	+	—	—	—	—
128.....	+	+	+	—	—
129.....	+	—	—	—	—
130.....	+	—	—	—	—
131.....	+	+	+	+	—
132.....	+	+	—	—	—
133.....	+	—	—	—	—
134.....	+	+	+	+	+

From the above table it will be interesting to note how some of the germs which seemed the least resistant to carbon dioxide are most resistant to the acid. No. 123 is a good illustration of this. In some of the other cases there is a correspondingly apparent inhibition due to the acid as was previously due to the carbon dioxide. Consequently some attention must be given to the direct bearing of the acidity upon the growth of micro-organisms in milk; accordingly a study of carbon dioxide in its action upon the indicator (phenolphthalein) should be made before considering the direct bearing the acidity has upon aëration, for it is very evident that the acidity of milk may be most erroneously estimated if the indicator does not satisfactorily respond. The action of carbon dioxide upon phenolphthalein is well known.

XXIII. HOW CARBON DIOXIDE MAY INCREASE THE ACIDITY OF MILK TO PHENOLPHTHALEIN.

Two Hesse flasks containing milk were sterilized, one was allowed to remain in contact with the air and the other had passed into it carbon dioxide to complete substitution. The following brief statement gives the results:

Flask of milk sterilized and exposed to air gave in acidity 16° (.144 per cent).

Same milk sterilized in Hesse flask and carbon dioxide substituted for air gave 37° (.333 per cent.) acidity.

Both flasks were allowed to stand 48 hours before testing acidity, using phenolphthalein as indicator in both cases.

This study is purposely made as explanatory to what follows. Phenolphthalein is the most satisfactory indicator for measuring the acidity, yet the influence of carbon dioxide and of acid phosphates upon it should be constantly regarded in the consideration of this work otherwise misinterpretations may follow. In the case above there has been practically an increase of over .1 per cent acidity, yet further work indicates that there is a greater difference in the amount of acidity produced in carbon dioxide cultures as compared with that produced in air cultures than can be accounted for by the influence of carbon dioxide upon the indicator.

XXIV. HOW CARBON DIOXIDE MAY INCREASE THE ACIDITY OF MILK CULTURES OF SPECIFIC MICRO-ORGANISMS.

Bearing in mind that carbon dioxide and acid phosphates may in and of themselves increase the acidity from .2 per cent to .3 per cent, yet the table which is appended indicates in many instances an advanced increase beyond this limit; in other words, the acidity is greater than that which can be accounted for by the carbon dioxide alone, the acid phosphate being equal in both instances. However, care should be taken to allow in each case, at least .2 per cent for the possible increase due to carbon dioxide present in cultures. Perhaps other indicators might be found which would avoid the trouble experienced by the use of phenolphthalein, still cochineal, methyl orange, rosolic acid, and litmus were tried and the results were even more unsatisfactory. The table below in several instances will give such an increase in the acidity, that to account for it by the presence of carbon dioxide would apparently be fallacious. We have not been able to increase the acidity of milk .5 per cent simply by saturation with carbon dioxide as was done in the Hesse flasks in which the acidity was increased .189 per cent.

XXI.

HOW CARBON DIOXIDE MAY INCREASE THE ACIDITY OF MILK CULTURES OF SPECIFIC MICRO-ORGANISMS.

No. of micro-organism.	119.	120.	121.	122.	123.	124.	125.	126.
Acidity of air cultures.....	.054	.18	.72	.176	.029		.118	.137
Acidity of CO ₂ cultures.....	.36	.68	1.06	.622	.529		.515	.436

No. of micro-organism.	127.	128.	129.	130.	131.	132.	133.	134.
Acidity of air culture.....		.641	.173	.094	.580	Neut.	.036	.670
Acidity of CO ₂ culture.....		.659	.432	.486	.731	.522	.367	.720

The only conclusion that can be drawn from germs 120, 122, 123, 125, 126, 129, 130, 132 and 133 is that the acidity produced must be in part due to the conditions under which they were grown. I find this further proved by the use of hydrogen in place of carbon dioxide, although the results differ.

XXV. HOW HYDROGEN MAY ALTER THE ACIDITY OF MILK CULTURES OF SPECIFIC MICRO-ORGANISMS.

Here again we find that the acidity varies when the micro-organisms in question are grown under hydrogen but not exactly in the same way as carbon dioxide. The methods employed were identical with those used in studying the influences of carbon dioxide. The following table will indicate the influence of hydrogen:—

TABLE XXII.

HYDROGEN ANAEROBIC CONDITIONS AS COMPARED WITH AEROBIC CONDITIONS REGARDING THE ACIDITY PRODUCED.

No. of micro-organism.	119.	120.	121.	122.	123.	124.	125.	126.
Acidity of air cultures.....	.098	.518	.472	.522	.284	.796	.288	.576
Acidity of hydrogen cultures..	.085	.63	.461	.551	.292	.418	.299	.706

No. of micro-organism.	128.	129.	130.	131.	132.	133.	134.	Control.
Acidity of air cultures.....	.742	.112	.220	.680	.212	.086		.162
Acidity of hydrogen cultures.....	.518	.209	.310	.641	.277	.166		.162

XXVI. IS IT THE CARBON DIOXIDE WHICH CAUSES A CONSTANT RISE OF ACIDITY IN CONFINED MILK IN CONTRA-DISTINCTION TO MILK EXPOSED TO THE AIR?

In this test, tubes were completely filled with milk, plugged so as to exclude all air, and the stoppered ends were placed in mercury. The milk used was from a sample obtained immediately after milking. The tubes and stoppers were sterile. Some of the same sample was placed in a Fernbach flask so as to form a layer 1 cm. thick. The acidity of the milk was 16° or .144 per cent. (estimated as lactic acid). The milk remained confined and also exposed for 16 hours. The summary of the work is as follows:

Milk confined for 16 hours gave 16° (.144 per cent.) acidity.

Milk exposed for 16 hours gave 13.5° (.1215 per cent.) acidity. (In tin vessel.)

Confined milk run drop by drop over six foot tin surface gave 14.5° (.1305 per cent.) acidity, a reduction of 1.5° (.0135 per cent.) in the acidity.

Confined milk brought to a brisk boil gave 14.5° (.1305 per cent.) acidity, a reduction of 1.5° (.0135 per cent.) in the acidity.

Here we have an indication that carbon dioxide accounts for at least 1.5° acidity, consequently a study of the acidity in confined milk without the influence of any gas has been undertaken.

XXVII. INFLUENCE OF CONFINEMENT OF MILK UPON ACIDITY.

The milk samples of these tests, the results of which are given in tabulated form, were, in each case, carried out in exactly the same manner as in the preceding experiment; that is, the milk confined and also that exposed to the air came from the same lot which was procured immediately after milking. In no instance was air allowed to remain in the tubes. If we go back and recall the gas-content of milk as found to exist immediately after milking the percentage of carbon dioxide would be about sixty. If carbon dioxide in the free state is the determining factor then there should exist corresponding results in acidity, likewise in the number of germs involved in the fermentation.

TABLE XXIII.

INFLUENCE OF CONFINEMENT OF MILK UPON THE ACIDITY.

(Numbers represent percentages calculated as lactic acid.)

TEST I.

	Confined.	Exposed over glass.	Exposed over tin.
After milking.....	.1125	.1125	.1125
After 21 hours.....	.1125	.072	.054
After 39 hours.....	.1305	.072	.054
After 48 hours.....	.1395	.035	.045
After 87 hours.....	.162	.0675	.195

TEST II.

	Confined.	Exposed over glass.	Exposed over tin.
After milking.....	.1215	.1215	.1215
After 20½ hours.....	.126	.09	.09
After 24 hours.....	.135	.09	.09
After 40 hours.....	.144	.117	.1385
After 48 hours.....	.144	.126	.225
After 64 hours.....	.144	.198	.315
After 69 hours.....	.198	.2295	.342
After 88 hours.....	.1755	.318	.486
After 96 hours.....	.1755	.423	.513

TEST III.

	Confined.	Exposed over glass.	Exposed over tin.
After milking.....	.135	.135	.135
After 4 hours.....	.162	.162	.144
After 8 hours.....	.1625	.144	.135
After 13 hours.....	.171	.144	.144
After 18 hours.....	.180	.180	.405
After 26 hours.....	.495	.4365	.531
After 34 hours.....	.7875	.666	.693

These tables are fairly representative of the results obtained after a great many tests. Usually the milk confined does not fall in acidity from the time when it is put up to the time when it begins to increase in acidity. Occasionally it will fall slightly. In the case of exposed milk in Fernbach flasks of glass there is a falling off in acidity for a time, then there follows a rapid increase until the milk completes its full fermentation. It is interesting also to note that in the case of a tin vessel made in the form of the Fernbach flask the falling off in acidity is much more noticeable; and usually after the acidity begins to increase, this increase is much more rapid than in the case of the glass Fernbach flask. These tables also show that usually the milk exposed to the air under the conditions given sours more rapidly than when confined; occasionally, but seldom, as in Test III, the reverse is the case. I have inserted this test as one that is somewhat rare. Tests I and II represent the usual results obtained. In connection with the acidity of milk, several problems present themselves which cast some light upon its gas-content. Unærated milk containing 60 per cent. carbon dioxide does not act the same as milk which has had 60 per cent. carbon dioxide maintained over its surface and with which it was mixed. We find that milk after milking has about 60 per cent. carbon dioxide which we estimate as free carbon dioxide and this will give a low estimate of acidity when phenolphthalein is used as an indicator, in fact the acidity is regarded as normal, but as soon as the free carbon dioxide is passed into the milk or the same percentage maintained over it immediately the acidity ascends rapidly. This condition may be explained by the solubility of carbon dioxide. Normal milk cannot be considered saturated with this gas. By passing carbon dioxide into the milk the acidity to phenolphthalein rises from 13° (.117 per cent.) acid to 19° (.171 per cent.) in ten minutes, to 28° (.252 per cent.) in thirty minutes, to 38° (.242 per cent.) in seventy minutes. This does not correspond in any manner to confined milk other than the fact that confined milk does not fall in acidity until fermentation sets in.

XXVIII. DO UNAERATED CONDITIONS OF MILK TEND TO HOLD BACTERIA IN CHECK?

In these tests, three of which are recorded as representative, I have endeavored to study the germ content in milk subjected to exactly the same conditions as we found in the previous study of acidity. The same sample of milk was used in each instance and great care exercised in securing exact dilutions and measures. In each count several plates were made from each sample and the average taken.

TABLE XXIV.

DO UNAERATED CONDITIONS OF MILK TEND TO HOLD BACTERIA IN CHECK?

TEST I.

	Confined.	Exposed over glass.	Exposed over tin.
After milking.....	8,400	8,400	8,400
After 21 hours.....	31,950	336,300	14,096,400
After 39 hours.....	756,000	84,292,500	125,650,500
After 48 hours.....	1,096,000	215,205,000	Curdled. 250,739,000
After 87 hours.....	1,747,500,000	8,142,500,000	10,037,500,000

TEST II.

	Confined.	Exposed over glass.	Exposed over tin.
After milking.....	6,620	6,620	6,620
After 20½ hours.....	31,850	46,750	331,400
After 24 hours.....	35,950	62,250	798,150
After 40 hours.....	360,000	53,282,000	914,895,000
After 48 hours.....	773,000	109,999,000	Curdled. 1,069,205,000
After 64 hours.....	160,000,000	877,500,000	3,722,500,000
After 69 hours.....	155,000,000	2,570,000,000	1,702,500,000
After 88 hours.....	112,500,000	1,760,000,000	1,650,000,000
After 96 hours.....	75,000,000	1,517,500,000	1,227,500,000

TEST III.

	Confined.	Exposed over glass.	Exposed over tin.
After milking.....	1,280	1,280	1,280
After 4 hours.....	4,660	5,120	9,400
After 8 hours.....	76,950	557,950	1,236,750
After 13 hours.....	2,779,300	15,582,000	21,036,500
After 18 hours.....	41,105,750	66,301,750	90,782,560
After 26 hours.....	333,939,000	454,522,000 curdled.	724,632,000

In the above tables it will be very apparent that the confined conditions of milk tend to reduce the number of bacteria. Milk exposed to air far exceed the number of germs in unexposed milk. Compare too the milk exposed over glass and the milk exposed over tin and the tin receptacle seems to favor the development of bacteria. This corresponds very closely to the previous tables in which a study of the production of acidity has been made. Why tin under these conditions favors the development of bacteria over the development of bacteria in a glass receptacle, unless the tin combines with carbon dioxide and also reduces the amount of lactic acid, is difficult to explain inasmuch as there appears to be some germicidal action instigated by bringing media in contact with metals. One factor, however, remains. In the case of tin we have a dark vessel and in the case of glass the entrance of light rays, although in a semi-dark room, may account for the reduced numbers in the glass vessel below those in the tin vessel. This hardly seems credible, however. The explanation will probably be found in the affinity of the tin surface for carbon dioxide and lactic acid.

An attempt was further made to ascertain whether by growing the plates, made from the milk in confinement, in hydrogen would increase the number of colonies developing. Several tests thus made only served in reducing the number of colonies instead of increasing them. We can understand this decrease to be due to the action of hydrogen.

XXIX. DOES AERATION INFLUENCE THE GERMICIDAL ACTION OF MILK?

This interesting action of milk should not be passed over without showing the possible influence aëration may have upon it. The impetus given to this work by Conn and Hunziker led me to attempt to ascertain whether milk unexposed to the air would have a greater or less germicidal action than milk exposed to the air. Several tests were made, six of which are recorded below giving the results in detail. The milk was secured with the apparatus used in study of the gas-content of milk without exposure to air.

TABLE XXV.

DOES AERATION INFLUENCE THE GERMICIDAL ACTION OF MILK?

No. of micro-organism.	I.		II.		III.	
	Aërobic.	Anaërobic.	Aërobic.	Anaërobic.	Aërobic.	Anaërobic.
After milking.....	2600	2600	3290	3290	3120	3120
3 hours after milking.....	520	680	2580	2080	1700	1440
6 hours after milking.....	790	730	1660	1430	1560	1310
9 hours after milking.....	610	850	1190	1270	1670	1210

No. of micro-organism.	IV.		V.		VI.	
	Aërobic.	Anaërobic.	Aërobic.	Anaërobic.	Aërobic.	Anaërobic.
After milking.....	3090	3090	12348	12348	11582	11582
3 hours after milking.....	620	920	8801	509	8070	10030
6 hours after milking.....	240	1090	3320	408	4840	9660
9 hours after milking.....	720	1160	3314	331	4490	4030

There is only one conclusion to be drawn from the above tests, although there are some results which do not fully accord: there is no difference existing between the germicidal action of unexposed milk and milk exposed to the air. Thus as far as the germicidal action is concerned we cannot consider that aëration favors it.

XXX. DOES AERATION AS PRACTICED REDUCE THE NUMBER OF BACTERIA WHILE THE MILK IS FLOWING OVER THE AERATOR?

We were not able to employ a common milk aëerator for this work but resorted to the use of glass, tin and copper sheets which we used for aërating purposes when studying the action of aëration upon the gas-content. The same inclined surfaces were employed and aëration was carried out in exactly the same way. The number of germs before aëration was estimated; the milk was run over the inclined sterile surfaces of the materials mentioned and then the number of germs estimated again. In the following table will be found detailed results:—

TABLE XXVI.

DOES AERATION AS PRACTICED REDUCE THE NUMBER OF BACTERIA SIMPLY WHILE THE MILK IS FLOWING OVER THE AERATOR?

Date.	Jan. 3d.	Jan. 4th.
Aërating surface.....	Glass.	Glass.
Extent of surface.....	6 ft. x 1½ in.	6 ft. x 1½ in.
Amount of milk aërated.....	1 L per hour.	1 L per hour.
No. of bacteria in unaërated milk.....	*2860	3630
No. of bacteria in aërated milk ..	2750	3710

*The number of bacteria found in 1 c. cm.

Jan. 6th.	Jan. 7th.	Jan. 8th.	Jan. 9th.
Glass.	Tin.	Tin.	Copper.
6 ft. x 1½ inch.	6 ft. x 1½ inch.	6 ft. x 1½ inch.	6 ft. x 1½ inch.
1 L per hour.	1 L per hour.	1 L per hour.	1 L per hour.
8410	2810	3100	2750
9590	2600	3310	3800

If this method of aëration can be regarded as one simulating the methods in vogue we are forced to conclude that the simple act of aëration does not reduce the number of micro-organisms in the milk and that if any reduction occurs it must be due to some indirect action which aëration must have upon the milk.

XXXI. SUMMARY.

- I. Milk drawn from the udder of a cow contains a high percentage of carbon dioxide and a low percentage of oxygen. The gas pumped from milk contains on an average 81.49¾% of carbon dioxide and 2.42% of oxygen.
- II. During the milking process the percentage of carbon dioxide contained in the pumped gases from milk dropped to an average of 59.63¾% and the oxygen increased to an average of 13.17¾%.
- III. During aëration the carbon dioxide of the pumped gases dropped still farther to an average of 40.57 1-6% over glass, 35.832% over tin, 42.328% over copper, and to 25.805% through glass wool; the oxygen increased to an average of 20.586% over glass, 20.55% over tin, 17.256% over copper and through glass wool to 20.805%.
- IV. Air confined over sterilized milk indicates no interchange of gases.
- V. Most micro-organisms in milk generate carbon dioxide and absorb oxygen.
- VI. Milk fresh from the cow and confined in a flask consumes the oxygen and liberates carbon dioxide upon fermentation.
- VII. When antiseptics are used in fresh milk, oxygen is very slowly absorbed and no appreciable quantities of carbon dioxide are given off.
- VIII. Free carbon dioxide in amounts of 100%, 96.5%, and 62.9% have a marked restraining action on bacteria and in some cases an inhibitive action.
- IX. A percentage of 32.9% of carbon dioxide stands on a border line where the action of this gas gradually disappears.
- X. When free carbon dioxide is made to act upon sterilized and inoculated milk the restraining influence is also very noticeable.
- XI. When bacteria are grown in bouillon under the influence of carbon dioxide, this restraining action is very prominent.
- XII. Free carbon dioxide has a direct influence upon the character and rate of milk fermentations induced by specific micro-organisms.

- XIII. Carbon dioxide in its free condition corresponds very closely in its action upon bacteria with carbon dioxide as it exists in milk, and it constitutes about the same percentage of the pumped gases.
- XIV. Hydrogen has practically the same action on bacteria as carbon dioxide.
- XV. The nitrogen or residual gas resulting from the absorption of oxygen from air by means of alkaline pyrogallate is not so detrimental to the development of bacteria as free carbon dioxide or hydrogen.
- XVI. Lactic acid has a varying influence upon the development of different micro-organisms.
- XVII. The acidity of milk is influenced by the aerobic and anaerobic conditions under which micro-organisms are cultivated.
- XVIII. Confined milk does not show a decrease in acidity after milking.
- XIX. Milk exposed freely to the air decreases in acidity for a few hours after milking then the acidity rises rapidly.
- XX. Carbon dioxide is one factor which keeps up the acidity of confined milk with phenolphthalein as an indicator.
- XXI. Bacteria do not thrive so well in unaerated milk without exposure as in exposed milk.
- XXII. The germicidal action of milk is not influenced by the aerobic or anaerobic conditions.
- XXIII. The aeration of milk does not reduce the number of bacteria directly.

A review of the summary immediately brings before us striking evidence that underlying aëration of milk are to be found indisputable data fundamentally important which may lead us to definite conclusions. In the determination of the gas-content of milk, the rapid decrease of carbon dioxide and the increase of oxygen content at once demonstrates that there is a considerable change in the gases of milk through aëration. There is to be no sanitary significance attached to this interchange of gases per se, but the fact that such a striking change is taking place is of sufficient weight to assist in the solution of pertinent problems.

We have not positively shown that these gases which have been subjected to study are in loose combination or free but since the carbon dioxide runs down rapidly to a certain percentage where its liberation from the milk becomes quite difficult and since the percentage of oxygen may be increased above the content of air, there does seem to be some evidence favoring the idea that there is a combination rather than the existence of the gases in the free condition, yet small amounts of carbon dioxide are with difficulty removed from distilled water and when such salts as sodium carbonate, sodium hydrogen phosphate et cetera are in solution this difficulty is greatly increased. Furthermore, when an attempt is made to simulate the effects of carbon dioxide in milk by the use of free carbon dioxide there exists a marked similarity so far as the growth of the germs is concerned, but just as soon as we begin to study the acid effects upon the indicator we find that the carbon dioxide which we obtain does not act the same as the carbon dioxide as we add it in the free state. However, it should be remembered that the solubility of carbon dioxide in water is practically 100 per cent. while the most obtained from milk is not above 10 per cent. by volume. It would be unsafe, therefore, to draw a positive conclusion from the facts we have

regarding the condition of carbon dioxide in milk or the condition of oxygen; our knowledge must remain as it is. The weight of evidence does not favor an apparently loose combination, because it may be accounted for by the solubility of the gas, and thus must at first follow a rapid reduction of carbon dioxide in the milk when exposed in an effort to establish an equilibrium.

The increase of oxygen during the milking process also must have considerable influence upon our judgment relative to the common methods of handling milk in the stable. If carbon dioxide is eliminated so rapidly and oxygen increases, there must of course be a rapid interchange of gases going on during the milking process while the milk is more or less agitated by the passage of the streams of milk from the teats of the cow to the surface of the milk in the pail, and to the impinging of the streams against the surface of the milk in the pail causing a churning effect. Now if this interchange takes place as is indicated by the analyses over and over again, it follows that if any obnoxious gases exist in the stable they must necessarily invade the milk as does the oxygen; by this means the milk is rendered unwholesome to a greater or less degree by the gases existing in the stable. Should the operations which are commonly in vogue at the present time be carried out and the milk disturbed by pouring it through a strainer into a milk can or even over an aëerator or cooler in the stable air it is easy to understand how the gases of the milk and the gases of the air find an equilibrium. This of course means that the gases in the milk and the gases of the air must substitute each other to a great extent. The practice of milking in filthy stables containing filthy odors is therefore reprehensible.

There may be an elimination of taints which originated by means of physiological processes in the cow or emanate from bacterial fermentation processes. It has been an old and common belief and is still that animal odors and taints are to a large extent removed by aëration. This is evidently true. In our experience we have so removed the animal odor from the milk that no odors were perceptible even when warmed. Knowing that the gas-content of milk and the air gases interchange freely when milk is agitated or spread out in a very thin film the explanation of this removal of animal odors and taints appeals to our understanding, and very naturally, in the light of preceding studies. However, the methods employed for aëration must be effective to accomplish satisfactory results.

The interchange of gases going on in a closed can of milk over which is a small air space as is usually the case should be looked upon as resulting from fermentation processes rather than from physical or chemical interchange of gases. Blyth has satisfactorily shown that milk undergoing fermentation soon reaches the point where oxygen completely disappears and carbon dioxide is practically the only gas present. In connection with antiseptics in fresh milk where all fermentation has been arrested it is true the oxygen greatly diminishes in amount, but it requires some time to bring about a marked diminution of this gas, consequently the interchange of gases is due to a slow oxidation process such as is well known to exist in a great many complex organic compounds; what oxygen may be present in the milk itself may be utilized by numerous possible bacterial changes. It is apparently true that if milk remains

quiet that the oxidation which takes place through contact with the oxygen in the milk is probably as slow as is known to be the case where oxygen permeates agar or gelatine and influences the life zones of the media. We should not expect that where milk is standing quiet that the oxygen diminution would be very noticeable for several hours even though fermentation be held in check. The production of carbon dioxide in large amounts by bacteria, the absorption of oxygen or the consumption of oxygen by bacteria have long been known.

Carbon dioxide does not exert any effect upon the germicidal action of fresh milk, since it is found that the germicidal action of milk confined without exposure to the air and that which is exposed to the air are practically the same; that is, the ratio of reduction of the number of germs does not differ in the two cases sufficiently to warrant us in drawing any other conclusion.

When the number of bacteria is estimated before and after the aëration of milk there is no appreciable reduction, consequently, we cannot look upon aëration as directly influencing the number of bacteria.

It is true that in confined milk germs do not increase as rapidly as in exposed milk but this simply supports the idea that the carbon dioxide or other anaërobic factors exert some influence over the development and increase of bacteria. Without entering into a demonstration of whether the confined conditions or anaërobic conditions of milk are likely to give rise to obnoxious fermentations we have regarded it more feasible to consider only the well established facts already known regarding aërobic and anaërobic conditions as suited to the productions of proper fermentations. Under aërobic conditions usually harmless fermentations take place; that is, fermentations confined to the ternary compounds consisting simply of carbon, hydrogen and oxygen occur, and in the destruction of these ternary compounds as is effected through these fermentations, oxygen must be present in sufficient quantities to yield the desired results. This is not always true, however, but may be regarded as generally true. Of milk fermentations, the ordinary lactic acid fermentation, the fermentation sought, is accelerated by the presence of oxygen, and if such fermentative processes may be facilitated by its action, it follows that harmful fermentations which are capable of development may be held in check. Usually where toxic or harmful effects are obtained the nitrogen molecule is acted upon and broken down. During this destruction toxic substances are formed thus producing the poisonous symptoms when the food is consumed. If a review is made of the various cases of poisoning from food, the greater number will be found to result from anaërobic or partially anaërobic nitrogenous decompositions. Brieger has shown that a trace of oxygen is necessary to the production of poisonous ptomains, but where there is a bountiful supply of oxygen no toxic ptomains are produced. Now, if it is true that the ternary compounds may be decomposed more readily in the presence of oxygen and that by this process the decomposition of the proteids may be held in check the value of substituting oxygen for carbon dioxide is plain. In the case of milk, lactic acid is depended upon to hold in check other fermentations. Repeating, lactic acid fermentation is facilitated by the presence of oxygen, consequently an actual supply of oxygen is required to control the possible detrimental fermentations. Should the milk be

practically free from bacteria the need for this control is not so apparently great as where considerable filth has entered the milk, making the possible number of harmful fermentations much greater.

CONCLUSIONS.

1. Milk undergoes aëration when exposed to air from the time it leaves the milk-duct of the cow until it is consumed or made into milk-products.

2. This aëration is demonstrated by the reduction in the amount of carbon dioxide and the increase in the amount of oxygen.

3. It has been shown that aërating methods which increase the surface of the milk exposed, facilitate aëration greatly.

4. Below a certain per cent the elimination of carbon dioxide becomes very difficult.

5. By this interchange of gases between air and milk, there is a great opportunity offered for the absorption of noxious gases by the milk, unless the interchange takes place in absolutely pure air.

6. Agitation of milk favors the interchange of gases, because of the increased surface exposure.

7. Odors and taints resulting from aromatic foods, physiological processes, and disease processes may be greatly reduced permanently.

8. Odors and taints resulting from bacterial fermentations may be greatly reduced, but they will return upon the further development of bacteria.

9. The oxidation of milk is a slow process in the presence of anti-septics.

10. Where the oxygen disappears rapidly and carbon dioxide is formed in a confined air space over milk, this change is evidently due to bacterial activities.

11. Closing milk cans from the air consequently reduces the amount of oxygen supply, therefore must change the conditions of germ-life.

12. Aëration does not change the germicidal action of milk.

13. Aëration does not reduce the number of bacteria.

14. Directly confined milk does not ferment more readily than aërated milk.

15. Aëration does influence the amount of oxygen supply to the bacteria present.

16. Mere traces of oxygen favors the development of toxic products by bacteria (Brieger).

17. A plentiful supply of oxygen causes bacteria to produce non-toxic substances (Brieger).

18. The fermentations of milk are therefore influenced by aëration, favoring the production of non-toxic substances.

In closing this article it is a great pleasure to state that I have had the privilege of freely consulting Prof. F. G. Novy as this work has progressed. I am also grateful to Prof. M. Gomberg for many valuable suggestions, and to Mr. Alfred White for his useful hints in gas analysis.

